MANAGEMENT IMPACTS ON THE QUIESCENCE AND SPROUTING OF SUBTERRANEAN TURIONS OF DIOECIOUS HYDRILLA
[*Hydrilla verticillata* (L.f.) Royle]

By

MICHAEL D. NETHERLAND

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In memory of Edwin Lane Netherland Sr

I wish I had had a chance to know you better

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By

Michael D. Netherland

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A greater understanding of the factors influencing sprouting and the population dynamics of subterranean turions (tubers) of hydrilla is critical to developing improved management programs for this invasive, exotic aquatic plant. Vertical distribution of hydrilla tubers in lake hydrosoils was determined, however, subsequent studies indicated that sprouting was not influenced by location of tubers in the sediment. Mesocosm studies (900 L tanks) indicated that simply removing the vegetative canopy of hydrilla does not impact tuber sprouting, however, control methods that kill the root system increased sprouting rates by 20 to 48 percent (independent of tuber age). Tuber sprouting was much greater in sand than in organic or loam sediments following treatment. Changes that occur in the microenvironment where roots and tubers are closely associated, likely stimulated sprouting in mesocosm studies. Studies of tuber population

dynamics over a 30-month period in research ponds in North Florida showed no difference in sprouting between untreated control ponds and treated (vegetation removed) ponds. Sprouting rates generally remained below 3 percent, with peaks (5-7%) noted in the fall. Limited tuber production in untreated systems was attributed to reduced rootcrown density (loci for tuber production) due to intraspecific competition. When management was stopped at 27 months, tubers were replenished to near pretreatment densities within 3 months. Laboratory studies show that once a tuber is disturbed following its removal from the sediment, the likelihood of sprouting increases linearly with time through 48 hrs. Use of disturbed tubers in laboratory studies may confound results depending on the length of time the tuber has been removed from the sediment. Laboratory evaluations suggested that exogenous application of abscisic acid at concentrations as low as 0.05 to 1.0 μM strongly inhibited tuber sprouting under both aerobic and anoxic conditions, however, this effect was partially overcome by addition of GA_3 (15-150 μM). Inhibitors of ethylene action and synthesis, as well as ethanol did not impact tuber sprouting at physiological concentrations. Carbon dioxide at concentrations of 1 to 14 atm also inhibited tuber sprouting. Results suggest that drawdowns remain the only management tool currently available that will significantly stimulate sprouting of hydrilla tubers.

CHAPTER 1
INTRODUCTION

Discovered in Florida in 1959, the exotic submersed plant hydrilla (*Hydrilla verticillata* (L. f.) Royle) has become a serious weed problem in Florida and many other states (Blackburn et al. 1969, Haller 1976). Problems associated with excessive aquatic plant growth are well documented and include both economic loss due to interference with water uses (irrigation, flood control, navigation, and recreation) as well as ecological consequences that result when a non-native plant displaces native aquatic plant communities and adversely impacts freshwater habitats. Due to its rapid expansion and ability to dominate entire aquatic systems, the biology and potential control for hydrilla have been extensively studied. Nonetheless, hydrilla has continued to expand, and control of this exotic species with either sterile triploid grass carp (*Ctenopharyngodon idella*) or herbicide applications remains both costly and controversial.

Due to its specialized growth habit, physiological characteristics, and various means of vegetative reproduction, hydrilla has been described as "the perfect aquatic weed" (Langeland 1996). Hydrilla is extremely competitive due to its ability to grow rapidly to the water surface and form a dense canopy that excludes light and prevents competition. Rapid hydrilla growth and expansion is favored by its low light and CO_2 compensation points, reduced photorespiration due to C_4-like photosynthetic metabolism, and prolific reproductive capacity (Haller and Sutton 1975, Van et al. 1976, Salvucci and Bowes 1983, Magnuson et al. 1997). Following formation of the dense surface canopy

these unique physiological traits enable hydrilla to survive the harsh conditions (high temperature, wide pH variation, reduced light and low CO_2) that result from dense canopy formation.

Hydrilla is very difficult to control due to its rapid growth potential and ability to reproduce from fragments, stolons, axillary turions and subterranean turions (Langeland 1996) It may be one of the most studied of all aquatic plants, yet basic understanding of the longevity, sprouting and quiescence of subterranean turions *in situ*, and in response to management practices remains limited

Hydrilla was recently reported to cover over 40,000 ha of water in 43% of the public lakes of Florida (Langeland 1996) Due to the continued expansion of hydrilla, the Florida Department of Environmental Protection recently initiated a program to bring this exotic submersed plant hydrilla under maintenance control in the large public water bodies in the State of Florida This program was instituted in 1996 and will lead to the expenditure of an estimated 30 to 50 million dollars This effort is modeled on the past success of bringing the exotic floating plant water hyacinth (*Eichhornia crassipes* (Mart) Solms) under maintenance control in Florida Maintenance control of water hyacinth required substantial herbicide treatments of large infestations (this requires both high initial chemical input and cost and high manpower requirements) and subsequent intensive management of these areas through low chemical input (high manpower requirements) These high maintenance programs prevent the exotic plants from reaching a threshold population that threatens to dominate an aquatic system Due to differences in the life histories of water hyacinth and hydrilla, maintenance control of hydrilla may

require a greater knowledge of the reproduction biology of hydrilla and different control strategies than are currently used for maintenance control of water hyacinth

Recent research has shown that the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone) can be used effectively at rates as low as 2 to 10 µg/L for control of hydrilla. This low rate technology and the slow mode of action of fluridone has allowed aquatic managers to economically and with minimal environmental impact, treat several thousand hectares (ha) of hydrilla in one growing season. While vegetative regrowth from subterranean turions (hereafter called tubers) of hydrilla is thought to be likely following these control efforts, the influence of intense management on subsequent tuber sprouting rates and tuber population dynamics in general is currently unknown. Greater knowledge of the sprouting dynamics of tubers will determine whether or not a conventional maintenance control concept is possible for hydrilla.

If hydrilla management (herbicides, mechanical, or biological means) stimulates a large number of tubers to sprout and treatments can be applied to prevent further tuber formation, then long-term management programs can be formulated. If tuber sprouting is generally random and non-seasonal following treatments, the concept of maintenance control as currently practiced on other plants may not prove successful. For example, if sprouting is generally random, tubers will form new vegetative growth throughout the lake allowing rapid restoration of hydrilla populations. It is important that sprouting dynamics of tuber populations are better documented in order to allow aquatic managers to devise the most efficient, environmentally sound and cost-effective measures to manage hydrilla.

Review of Pertinent Literature

Despite extensive control efforts during the past 35 years, the exotic macrophyte hydrilla remains the dominant submersed weed problem in the southeastern United States (US), and it continues to spread northward. Perennation and spread of hydrilla by the asexual production of copious numbers of subterranean and axillary turions has received considerable research attention because these propagules represent the key target in breaking the life-cycle of hydrilla. These detached turions serve as a persistent meristem bank (analogous to a seed bank) that allows for re-infestation for an unknown length of time following applications of control techniques (Steward 1969, Haller et al 1976, Langeland 1993). Improving control strategies for hydrilla requires a better understanding of factors that influence propagule formation, quiescence, sprouting and longevity.

Background Information on Hydrilla

Cook and Lüönd (1982) wrote a taxonomic revision of the single species genus (*Hydrilla*) providing information on the ecology, floral biology, anatomy, chromosomes, genetics, and variation. The native range of hydrilla is uncertain, but evidence points to an origin in the warmer regions of Asia, although an origin in central Africa has not been discounted. A wide geographically disjointed distribution is reported, with hydrilla found on all continents except Antarctica (Cook and Lüönd 1982, Pieterse 1981).

Both monoecious (staminate and pistillate flowers on the same plant) and dioecious (staminate and pistillate flowers on separate plants) biotypes have been described, and both are present in the U.S. Cook and Luond (1982) report that on a worldwide basis the monoecious strain dominates in climatically tropical regions whereas the dioecious strains are largely temperate. However, the current distribution

and estimates of potential distribution for both monoecious and dioecious biotypes in North America are contrary to this observation The dioecious strain (female plants only), was first reported in the U.S in south Florida in the late 1950s, while the monoecious strain was first reported in the Potomac River in the mid-1980s (Steward et al 1984). A current distribution map is presented in Figure 1 The two biotypes have several differences in terms of vegetative growth habit and asexual propagule production

To conform to the majority of published literature, and to facilitate reading of this manuscript, the subterranean turion will be referred to as a tuber From a botanical standpoint, true tubers do not have leaf scales or leaves (Sculthorpe 1967), and are generally characterized by the swelling of a slender rhizome containing several buds (with undeveloped internodes) from which new growth arises. This is in contrast to the single apical meristem contained within the tip of a subterranean turion Axillary turions will be referred to simply as turions throughout the remainder of the text

Morphology of Hydrilla Tubers and Turions

Morphological descriptions of tubers and turions have been reported by Yao et al (1984) using light and electron microscopy, and by Mitra (1955) using light microscopy and line drawings Turions form in the axils of leaves or branches while tubers form at the terminal nodes of typically underground stems (rhizomes that can penetrate submersed soils up to 30 cm deep) that exhibit positive geotropism Turions and tubers are similar anatomically as both form when overlapping leaf scales and leaves surround a dormant plant meristem Turions appear as simple green compressed shoots, 3 to 12 mm in length (Lakshmanan 1951, Mitra 1964) Tubers are generally 4 to 15 mm long and can vary in color from off-white to near black. The basal two-thirds of the tuber are swollen

Figure 1 1 Reported distribution of hydrilla in the United States Dark gray shades marked with a D denote confirmed exclusive dioecious populations, light gray shades marked with an M denote confirmed exclusive monoecious populations States marked with M/D denote areas where a confirmed overlap of the monoecious and dioecious strains exist States marked with a ?- denote unconfirmed reports (not in peer reviewed literature) of presence of hydrilla in the state

and filled with starch (Miller et al 1976) The terminal one-third of the structure contains the apical meristem which bends at a 90 degree angle (Yao et al 1984) Spencer et al. (1987) reported that mean weights of propagules collected throughout the US ranged from 160 to 376 mg and 179 to 202 mg for dioecious and monoecious tubers respectively and from 36 to 77 mg for monoecious turions.

Both types of propagules become detached from the parent plant following formation. Turions of dioecious hydrilla develop an abscission zone and fall to the substrate in late autumn (Yao et al 1984) The time required for a tuber to become detached from the rhizome (parent plant) is largely unknown, but is likely enhanced by increased temperatures

The detached tuber has been described as tough and fleshy due to the fact that the leaf scales are several cells thick and a thick cuticle covers the external cell walls of the epidermis (Yao et. al 1984, Pieterse 1981) While the cuticle is usually described as highly reduced in eloeid species such as hydrilla (Sculthorpe 1967), no research has been conducted on the effects of the substantial cuticularization of the subterranean turion in relation to longevity, pest resistance, quiescence, and sprouting

Tuber and Turion Initiation and Formation

Many aquatic plants produce specialized propagules in order to survive conditions that are unfavorable for growth and to ensure vegetative reproduction (Sculthorpe 1967) Mitra (1955, 1956, 1960, and 1964) noticed the increasing presence and problems caused by the native plant hydrilla in the freshwater systems of India, and published a series of papers describing the autecology of hydrilla and the likely contribution of tubers and turions to its spread and continued dominance over other native submersed plants In

India, Mitra (1955) noted that hydrilla produced both tubers and turions beginning in November and continuing through March.

In the U.S., Haller et al (1976) reported that dioecious hydrilla formed tubers from October through April in Florida, whereas Harlan et al (1985) reported that monoecious hydrilla formed tubers from June through October in North Carolina. Subsequent work has shown that initiation of tubers and turions in dioecious hydrilla is primarily a response to short days. A critical day length of less than 13 hr is necessary for dioecious tuber and turion formation and warmer temperatures also increase tuber production (Van et al 1978). McFarland and Barko (1990) also reported that dioecious tuber formation was greatest under short days but could be stimulated during long days (14 hr) at lower temperatures (20 C). A recent report suggests that exposure of dioecious hydrilla to a minimum of 20 to 38 short days is required to induce tuber formation (Thakore et al 1997).

Recently, Spencer et al. (1997) reported that construction costs (g glucose g^{-1}) for tubers was similar to that for shoots. Therefore, during short days, tubers would be expected to be sinks for photosynthate that are nearly equal to, if not greater than shoots, and this may account for the decline in shoot biomass for hydrilla exposed to inducing conditions (Spencer et al 1994a).

The classic phytochrome-mediated and photoreversible system is involved in the initiation of tuber and turion production in dioecious hydrilla, with red light (660 nm) stimulation and far red (750 nm) repression (Klaina and Ward 1984). The dependence of dioecious tuber formation on photoperiod and the phytochrome system, has led to suggestions that night-interruption by a brief exposure to low level light (such as that

around boat marinas) could prevent tuber formation (Klaine and Ward 1984, Spencer and Anderson 1986)

Studies comparing the differential photoperiodic response between monoecious and dioecious hydrilla have produced some contrasting results. Spencer and Anderson (1986) reported the monoecious biotype grown from a tuber produced new tubers 28 to 56 days following a 10-12 hr photoperiod at 24 ± 0 3 C, but no tubers were produced during a 14 to 16 hr photoperiod The dioecious biotype did not produce tubers at any photoperiod tested The lack of tuber production by dioecious hydrilla reported by Spencer and Anderson (1986) at the shorter photoperiods is in direct contrast to several other studies (Van et al. 1978, Sutton et al 1980, Klaine and Ward 1984) However, Spencer and Anderson (1986) suggested that the use of shoot apices instead of tubers left open the possibility that the source plants used in other studies had already been induced to form tubers Subsequent work by Van (1989) in which tubers were used as the source tissue showed that the monoecious biotype produced new tubers after 28 d exposure to a 10 hr photoperiod and after 56 d exposure to a 16 hr photoperiod. Dioecious hydrilla formed tubers after a 56 d exposure to a 10 hr photoperiod, whereas no tubers were formed during the 16 hr photoperiod Furthermore both the monoecious and dioecious biotypes increased tuber production several fold when temperatures averaged 29 C compared to 21 C (Van 1989)

Although differences in study protocols likely influence eventual production of tubers, data from the studies conducted to date agree that monoecious and dioecious hydrilla respond to photoperiod in a differential manner Monoecious hydrilla is capable of forming turions and tubers under much longer photoperiods (up to 16 hr d) These

comparative studies also showed that monoecious hydrilla is more prolific in the formation of tubers and turions (2- to 7-fold greater) than dioecious hydrilla under similar conditions (Spencer et al. 1987, Steward and Van 1987, Van 1989 Sotton et al 1992). Sutton et al. (1992) used a single tuber for starting material and showed that monoecious hydrilla produced large numbers of propagules in both winter and summer in south Florida, whereas dioecious hydrilla showed a distinct seasonality with tubers produced only during the fall and winter It is unlikely that significant tuber production from monoecious hydrilla occurs in the late fall and winter in northern climates as vegetation dies in the winter and exhibits an annual growth habit (Harlan et al 1985)

Despite monoecious tuber production being more than 50% greater than that of dioecious hydrilla, the average weight of individual dioecious tubers was 32% greater than monoecious tubers (Sutton et al 1992). MeFarland and Barko (1987) and Spencer et al (1987) also reported that on average, monoecious tubers were significantly smaller than dioecious tubers, leading these authors to speculate that under field conditions the smaller monoecious tubers may not contain adequate starch reserves to survive as long as dioecious tubers.

Although overlap of monoecious and dioecious hydrilla in the same body of water has been reported in North Carolina and Virginia (Ryan et al 1995), for the most part, the genetically distinct biotypes (Verkleij et al 1983, Ryan et al 1991) continue to remain geographically separated. The initial gangraphic separation is likely due to different anthropogenic introductions, however, it has been related to the life history of the biotypes, and to potential vegetative reproductive success at varying latitudes Steward (1997) has recently evaluated several races of hydrilla and suggested that all races

produced tubers under short-day conditions, and that all monoecious races currently established in the United States appear capable (under proper temperature conditions) of tuber production throughout the year. Spencer and Anderson (1986) used a 9 C temperature cutoff and a 13 hr photoperiod for tuber production by dioecious hydrilla, and suggested that the monoecious strain may be better able to colonize more northerly areas due to its ability to form greater number of tubers in a shorter period of time. Van (1989) also noted the ability of monoecious hydrilla to form tubers under long summer days and temperatures which favor active growth. It was suggested monoecious tuber development in the summer would assure survival in the northern United States (Van 1989).

A recent report of a persistent population of dioecious hydrilla in Connecticut (Les et al. 1997), contradicted earlier speculation concerning the potential northward expansion of dioecious hydrilla. Although this report has not been refuted in the literature, some scientists suspect that this plant is actually the monoecious biotype (John Madsen personal communication).

To date studies addressing the competitive interactions and ability of the two biotypes to produce vegetative propagules under differing environmental conditions have not been addressed, largely due to the inability to visually distinguish the two biotypes. Distinguishing monoecious and dioecious tubers without the use of isoenzyme analyses is a significant impediment, however overlap in lakes of North and South Carolina may provide insight into the competitive success between these biotypes at different latitudes.

Information on production of turions is quite limited compared to that of tubers. Spencer et al. (1994a) noted that once the plant is initiated under a short photoperiod (11

b), carbon and nitrogen are directed from shoots and roots into newly formed tubers and turions, however, approximately 15 times more carbon and nitrogen were allocated to tuber production than to turion formation in rooted plants.

Miller et al (1993) report that in dioecious hydrilla, turion production in northern Florida begins under short days in September, decreases during cold months of the winter, increases again in late spring and essentially ceases during June through August Free-floating plants produced three times more turions than rooted plants, and the increased plant density resulted in decreased turion formation.

Thullen (1990) reported that dioecious turion production from floating plant fragments was influenced by daily temperature ranges, the source of the plants, the length of time the plants were in the study, and aeration Pieterse et al (1984) have suggested that turion formation is stimulated by low levels of nitrogen and phosphorous in the water Free floating plants would be much more subject to this stress as compared to rooted plants which receive the majority of these nutrients from the sediments However, Thullen (1990) concluded that turion production was not stimulated solely by low levels of nitrogen and phosphorous but required an adequate daily temperature range (17 to 27 C) and photoperiod

It is interesting to note that while the production of tubers is generally much greater than turion production in the US, Pieterse (1981) states that in Europe only axillary turions are formed by dioecious hydrilla. Similarly, Nakamura and Kadono (1993) report that in Japan the dioecious biotype produces only turions, whereas the monoecious biotype produces tubers. To date, no hypotheses have been proposed to explain these discrepancies in tuber and turion production

It has been hypothesized that the competitive ability of a plant is related to the size of propagules produced (Grace 1985). Spencer and Rejmanek (1989) evaluated the competitive abilities of tubers versus turions of monoecious hydrilla and concluded that the smaller turions produce plants that are weaker competitors. Spencer et al. (1987) have suggested that turions and tubers represent different survival strategies, with turions better suited for dispersal and possible occupation of non-vegetated areas where they are likely to face little competition. In contrast, tubers are not mobile and may need the extra storage reserves as they are more likely to face intraspecific competitive pressures. In support of this, Bowes et al. (1977) noted that larger dioecious tubers showed increased survival rates compared to smaller tubers when deprived of light for up to 4 months.

The monoecious and dioecious biotypes of hydrilla differ in many aspects of asexual reproduction and vegetative growth habit. Therefore management plans will likely require modification as these biotypes spread and begin to overlap in the U.S.

Quantification and Tuber Distribution

Spencer and Ksander (1993) have speculated that clonal species such as hydrilla would be expected to produce a clumped distribution of tubers versus a random or uniform distribution. Subsequent field sampling has supported this assertion. Haller et al. (1976) noted that following extensive sampling during a lake drawdown, core samples taken in the same location produced a high level of variability (0 to 12 tubers per 10 cm diameter core). Due to the non-random distribution and seasonal and site differences, numbers reported from field sampling are often quite variable and substantial replicate sampling is required to achieve meaningful values. The length of time a site has been infested with hydrilla or recent management practices may also affect tuber densities

within a given sample site Nonetheless, the history of hydrilla and recent management practices are generally not provided in reports Sampling techniques usually involve sediment coring devices similar to that described by Sutton (1982).

The production of millions of propagules per hectare following 2 5 years of hydrilla infestation led Haller and Sutton (1975) to suggest that control methods would be extremely difficult and competition from native aquatic plant species almost impossible Bowes et al (1979) noted large variations in tuber numbers between lakes in north and south Florida, and between time of season sampled. Sutton and Portier (1985) reported on the density of tubers of dioecious hydrilla in five south Florida lakes and showed significant differences between lakes and yearly differences within lakes but no distinct seasonal fluctuations were noted Subsequent work by Sutton (1996) at one of these sites has shown that differences in the number of hydrilla propagules collected occurred due to collection date, location, and site within the location sampled (interactions were noted between these three variables)

Harlan et al (1985) reported that field densities for monoecious tubers on three North Carolina lakes ranged from 200 to 1228/m² with no seasonal trends identified These authors also noted that generally 93 to 100% of monoecious tubers were located in the top 12 cm of hydrosoil Turion densities were low compared to tuber densities ranging from 0 to 42/m² Information on field densities of monoecious tubers is scarce compared to reports for dioecious hydrilla tubers

Miller (1975) suggested that tuber production increased with increasing water depths (up to 3 m), however, review of the research suggests that the shallow water sites (< 1 0 m deep) were periodically dominated with emergent and floating vegetation prior

to sampling. Mitra (1964) has reported that tuber density decreased with increasing water depth and MacFarland and Barko (1995) reported that monoecious tuber density and percent germination was greatest at approximately 1 m water depths (compared to 0.5, 1.5, and 2.0 m depths) in samples taken from the tidally influenced Potomac River. Nonetheless, to date, the role that water depth plays in either significantly reducing or increasing tuber production and/or sprouting and quiescence is largely unknown.

Difficulties in obtaining uniform core samples from different types of sediments and the high spatial variability of tubers results in a substantial sample variation associated with tuber sampling. Sutton and Portier (1985) reported that statistically valid results were obtained through the collection of 25 core samples (10 cm diameter) for each of 5 sample locations and 18 sample times. Spencer et al. (1994b) have evaluated several data sets and reported that when tuber density is low (<200 m^2) between 25 and 200 samples (10 cm diameter) are required to estimate tuber number to within 20% of the mean value, whereas, between 8 and 25 samples are required to estimate to within 20% of the mean value when tuber density is high (200-1000 m^2). Increasing the core diameter can decrease the number of samples required, but will increase the processing time per sample and the level of effort required to collect the sample.

To overcome problems with variability in the field, mesocosm studies (i.e. outdoor tank or pool) have been conducted to determine potential for tuber production. Sutton et al. (1980) reported on the intraspecific competition of hydrilla and found that the initial number of shoot tips planted significantly increased the number of tubers produced, whereas biomass remained the same regardless of the initial number of stems planted. Few studies have reported on turion production in field or mesocosm conditions

but Miller et al (1993) reported turion production for mats of detached dioecious hydrilla as high as 861 turions/kg fresh wt./month

Discrepancies between tuber values reported in the field and mesocosms have not been discussed in the literature. However, this difference appears to be anomalous, as vegetative biomass values are often similar between field and mesocosm studies Potential reasons for this discrepancy in tuber values may include the following (1) higher stem densities per unit area (more loci for tuber formation) in mesocosm chambers (Sutton et al 1980), (2) optimal growth conditions (limited competition and herbivory, adequate nutrients, and copious light for photosynthate production) in mesocosms, (3) shallow and uniform depth of the mesocosms; and (4) different rates of tuber death and/or sprouting in the mesocosms. Sutton and Portier (1985) suggest that under field conditions, tuber density may reach a steady state in which formation of new propagules equals those sprouting (and those dying and decaying) with the maximum number for a body of water dependent on sediment type and nutrition, water quality, and unknown factors. In contrast, mesocosm studies are set up to determine maximal tuber production in a short time period, and studies are likely terminated before a steady state is attained.

Bruner and Batterson (1984) concluded that tuber formation was independent of soil type (sand, marl and potting mix) and was an intrinsic property of the plant, however, these authors suggested that soil fertility influences tuber production In contrast, Sutton (1985) reported that although vegetative biomass was directly related to increased fertilization following a 16-week study, tuber production in a sand medium was independent of three levels of fertilizer It should be noted that both tuber and biomass production were reduced 8-10 fold in an unamended sand soil versus the fertilized sand

Steward (1984) compared sediment fertility and texture and concluded that increased fertility had a greater influence on vegetative biomass than on dioecious tuber production by hydrilla during a 70-week study. McFarland and Barko (1990) also report that while vegetative growth was reduced on sand- versus nutrient-amended sediment, dioecious tuber formation was unaffected by sediment type. In addition, Sutton and Portier (1995) noted that while sediments from six different Florida lakes supported different levels of shoot biomass, tuber numbers were not directly dependent on sediment type and were indirectly affected by the amount of shoot biomass the sediment would support. Nonetheless, the ability of sediments to support luxuriant plant growth is likely tied to substantial tuber production. One factor that may explain wide variability reported for field densities of tubers is the relationship between tuber production and the sediment type and fertility (Sutton and Portier 1995).

Spencer et al. (1992) reported that sediment type and organic amendments affected both tuber mass and number in monoecious hydrilla. Addition of a straw or peat organic amendment (5 to 20%) to any of the six substrates tested (sand, loam, 2 clays, silt-loam, and sand-clay-loam), resulted in increased tuber production. The authors speculate that addition of organic matter increased sediment nitrogen, leading to increased vegetative growth and subsequent tuber production.

Field sampling for hydrilla propagules remains difficult due to the random and clumped distribution of tubers, sampling difficulties associated with different sediment types and with blindly sampling sediments. Nevertheless, long-term management plans for hydrilla control must include tuber sampling in order to determine at what point a propagule bank no longer presents a viable threat of re-infestation.

Response to Abiotic, Biotic, and Anthropogenic Induced Stress

The formation of subterranean propagules not only ensures vegetative reproduction, but allows the plant to survive biotic, abiotic, and anthropogenic induced stress. Basiouny et al (1978b) reported that dioecious hydrilla tubers could survive and sprout following drying of up to 64 h at 30 C and 40% relative humidity. In contrast, turions only survived for up to 8 hr. The authors make no mention of it, but the thickened cuticle of the tuber compared to the turion likely enhances its ability to survive desiccation.

Dioecious tubers incubated in complete darkness over a 4 month period showed that increased survival and shoot length were directly related to initial tuber size, with larger tubers showing increased survival rates (Bowes et al. 1979). These authors suggested that a sprouting tuber must reach a quantum flux density of at least 12-20 $\mu E/m^2/sec$ within 0.5 to 0.75 m above the hydrosoil or it cannot survive.

Carter et al. (1987) examined the effect of salinity on sprouting of monoecious hydrilla tubers and concluded that a salinity of 0.3 parts per thousand (ppt) had little affect on sprouting, while 5-9 ppt resulted in only 4 to 20% sprouting and none sprouted at salinities greater than 9 ppt. Although there are no reports of salinity effects on sprouting of dioecious tubers, Steward and Van (1987) compared salinity tolerance of monoecious and dioecious hydrilla grown for 2 weeks from sprouted tubers and reported no differences up to 11 ppt, whereas, growth was severely retarded above 13 ppt.

Phenolic acid content of tubers and axillary turions of both hydrilla biotypes was investigated by Spencer and Ksander (1994), with values ranging from 6 to 20 $\mu M/g$ dw. The phenolic acid content is important because plant phenols often serve as defenses

against attack by microorganisms and herbivores Berhardt and Duniway (1986) noted a high incidence of propagule decay in drained irrigation canals and showed that three fungal isolates (*Fusarium sp Papulaspora sp and Geotrichium sp*) were able to colonize apparently healthy hydrilla tubers, reduce sprouting and increase decay rates in a subsequent laboratory study The susceptibility of tubers to pathogenic attack has received little attention, but the fact that the tuber normally resides in an anaerobic environment, reduces the potential for aerobic pathogens to play a significant role outside of drawdown situations Little, if any research has been conducted on seasonal tuber mortality and the role it plays in the stability of the propagule bank.

Godfrey and Anderson (1994) showed that insect feeding by *Bagous affinus* larvae can significantly reduce dioecious hydrilla tuber sprouting and suggested that *B affinus* should be released in the field (during drawdown or dry season) with an egg to tuber ratio of 2 1 or greater Nevertheless, in areas of high tuber densities this would require that several million insects be released per hectare Recent mesocosm studies by Van et al (1998) have suggested that feeding by the insect biocontrol agents *Hydrellia pakistanae* Deonier and *Bagous hydrillae* O'Brien reduced tuber production by as much as 43%, and resulted in shifts in the competitive balance between hydrilla and vallisneria due to selective insect feeding

Sutton (1986) has evaluated the effects of several potential allelopathic compounds on tubers and showed that sprouting could be greatly reduced by many of these compounds He concluded that with the exception of salicylic acid, the usefulness of these compounds in the management of hydrilla was limited due to the high concentrations required Sutton and Portier (1991) reported that two species of

Eleocharis applied as dried, ground material at rates of 5 to 10 g/kg of hydrosoil, reduced shoot dry weight and tuber production of hydrilla by greater than 80%. The authors attributed these significant reductions to phytotoxic allelochemicals released by the *Eleocharis*. The submersed arrowhead *Sagittaria subulata* grown in conjunction with hydrilla was reported to reduce tuber production by 59% (Sutton 1990), however, it was not determined whether the primary factor responsible was allelopathy or competition.

Spencer and Ksander (1995) noted that the microbial metabolite, acetic acid, applied to sediments at rates of 17 to 696 mmol/liter completely inhibited tuber sprouting at exposure times as short as one day. Little is reported about the effects of pH on tuber survival and sprouting, yet based on the wide variety of sediments in which hydrilla can grow, a fairly broad range of pH tolerance is suspected. Steward and Center (1979) evaluated the feasibility of using the fumigant metham (sodium methyl-dithiocarbamate) for control of hydrilla regrowth from tubers and concluded that subsoil injection at rates of 75 to 373 liters/ha of metham on moist soil, followed by leaching was the most effective treatment.

Steward (1980) evaluated 25 herbicides and found that the preemergence herbicides fenac [(2,3,6-trichlorophenyl) acetic acid] and dichlobenil (2,6-dichlorobenzonitrile) were the only compounds registered for use at that time that retarded sprouting or growth of shoots from tubers in laboratory studies. Subsequent pond studies showed that fenac at 1 to 2 mg/L inhibited hydrilla regrowth for 13 to 18 months, whereas, dichlobenil treatments (0.7 to 1.2 mg/L) resulted in regrowth comparable to controls in 6 to 8 months. Propagules collected from dichlobenil treated ponds readily sprouted and grew while those collected from fenac treated ponds did not

sprout and subsequently decomposed. Interestingly, no differences were noted between pretreatment and 13 month posttreatment tuber densities, suggesting a low rate of sprouting/death of tubers occurred during the year following treatment. Neither of these products currently have a Federal Aquatic Use Registration

Bensulfuron methyl (methyl 2-[[[[[(4,6-dimethoxy-2 pyrimidinyl)amino] carbonyl]amino]sulfonyl]methyl]benzoate) has been reported to significantly reduce or prevent tuber formation in both monoecious and dioecious hydrilla up to 6 months after treatment at rates of 50 to 200 µg/L for 28 exposure days (Van and Vandiver 1992, 1994). The authors suggested that proper timing of bensulfuron methyl (BSM) application would be critical to stopping vegetative reproduction due to differential seasonal tuber production by the monoecious and dioecious biotypes. Langeland and LaRoche (1992) reported that BSM at rates of 25 to 200 µg/L applied in either June or November completely inhibited dioecious tuber production during the following winter season. Haller et al. (1992) showed that BSM treatments as low as 5 µg/L could prevent tuber formation depending on the time of application and number of treatments. Anderson (1985) has suggested a growth regulator mechanism for prevention of tuber formation by BSM, as lower treatment rates often have little impact on vegetative biomass, yet they completely inhibit tuber production. Langeland (1993) reported on several lake treatments with BSM and concluded that even though there were large reductions in tuber numbers, high tuber densities (up to 300 m^2) remained in two of the lakes and tubers were not eliminated in any of the lakes up to 2 years after application. It was hypothesized that elimination of hydrilla tubers would be a long-term process that would likely require several years of annual sequential applications. Despite its excellent

ability to inhibit hydrilla tuber production (and potential sprouting), BSM is not currently being considered for aquatic registration.

MacDonald et al. (1993) showed that the currently registered herbicide fluridone applied at rates of 5 to 50 µg/L could also greatly inhibit tuber formation, and suggested the mode of action was due to decreased abscisic acid (ABA) formation. However, these studies also showed that at rates of 0.05 to 0.5 µg/L fluridone, young (but not mature) plants were stimulated to increase tuber formation, suggesting a growth regulator response. Miller et al. (1993) reported that fluridone and BSM reduced dioecious turion production at rates of 2.5, 5, and 10 µg/L.

Steward (1969) conducted laboratory evaluations on the effects of such currently registered contact herbicides as endothall (7-oxabicyclo[2.2.1] heptane-2,3-dicarboxylic acid) and diquat (6,7-dihydrodipyrido [1,2-a: 2',1'-c]pyrazinediium ion) on sprouting of hydrilla tubers and found these herbicides exhibited little or no phytotoxicity to quiescent propagules. It is highly unlikely that most herbicides ever come in contact with the tubers following a submersed application. However, due to its longer persistence and activity at extremely low concentrations (2-5 µg/L), fluridone has the potential to impact tuber sprouting, or show phytotoxicity to newly sprouted tubers. Although short residual contact herbicides do not reach the tubers, the resultant rapid removal of biomass has been reported to significantly stimulate *in situ* tuber sprouting (Van and Haller 1979, Joyce et al. 1992).

Growth regulating compounds have been shown to have stimulatory and inhibitory effects on both tuber initiation and germination. Klaine and Ward (1984) reported that application of exogenous ABA greatly stimulated turion production,

whereas addition of GA and ethylene (applied as ethephon) reduced turion production by 80%. Klaine (1986) showed that the compound thidiazuron (an ethylene stimulator) at a concentration of 10^{-6} M completely inhibited both tuber and axillary turion formation in dioecious hydrilla over a 227 day test period. MacDonald et al. (1993) suggested that inhibition of ABA production by the herbicide fluridone also reduces formation of tubers.

Steward (1969) reported that germination and growth of tubers was enhanced by gibberellic acid (GA), while that of axillary turions was enhanced by indole acetic acid (IAA) and 2,4-D. Sastroutomo (1980) noted that GA at 10^{-5} M broke dormancy of non-cold treated axillary turions, but was toxic to their development after germination. Tuber sprouting was also enhanced by ethephon, GA, and thiourea (Basiouny et al. 1978a). To date, hormone treatments have resulted in increased sprouting rates, however, there are no reports for application of ABA or ethylene inhibitors on hydrilla tuber sprouting.

Numerous chemical and non-chemical evaluations, have led to hydrilla management strategies that inhibit tuber production, however, the presence of large numbers of persistent underground propagules that are resistant to treatment continues to complicate hydrilla management.

Environmental Factors and Tuber and Turion Sprouting

Mitra (1956) provided the first accounts on the sprouting and autecology of hydrilla tubers and turions (India). She noted that tubers generally outnumber turions and could be found up to 18 cm deep in the sediment. Sprouting tubers are characterized by long internodes and pale rudimentary leaves until they reach the soil surface. It was reported that it generally takes about 12-14 days for the formation of a fully developed plant.

Haller et al (1976) reported that dioecious tubers and turions removed from the substrate showed optimum sprouting at 15 to 35 C, with low rates of sprouting (< 10%) noted below 15 and above 35 C. Steward and Van (1984) reported 35 to 68% germination rates for monoecious tubers exposed to 15 C, whereas, sprouting rates for dioecious hydrilla were only 3% at this temperature. Miller et al (1976) used light (12 µmol/m²/sec) to stimulate the rate of dioecious tuber sprouting; however light quality had no effect on sprouting percentage. Although light stimulated sprouting, a high percentage of tubers (63-68%) also sprouted under dark conditions during the 14 day incubation period. The role that light plays in stimulating *in situ* sprouting remains intriguing, as it seems highly unlikely that light could penetrate more than a few mm of sediment. Miller et al. (1976) also reported that a 100% CO_2 environment inhibited sprouting, whereas a nitrogen sparged medium (anaerobic conditions) had no effect on sprouting and recent studies showed that anoxia (nitrogen atmosphere) actually enhanced sprouting rates (Spencer and Ksander 1997).

Kojima and Iiswa (1989) reported that optimum conditions for tuber sprouting included soil moisture between 40 and 60%, temperature between 20 and 25 C, and <4 cm of overlying sediment. The authors reported that short periods of low temperature easily broke dormancy. Basiouny et al (1978a) also reported that maintaining winter-collected dioecious tubers at 5 C enhanced sprouting, whereas, summer collected tubers required no cold treatment. It should be noted that the authors were not able to distinguish between tubers that were formed within that season and those carried over from previous seasons and it is possible that some of the tubers used for the winter germination studies were not fully mature. Carter et al. (1987) showed that monoecious

tubers collected in the fall and chilled for 42 days at 7 C resulted in 92% germination, whereas propagules that were not chilled failed to germinate. In contrast, Harlan et al. (1985) showed a high percentage of germination of monoecious tubers (>85%) stored at 26 C in the laboratory. Sastroutomo (1980) reported that axillary turions of monoecious hydrilla germinated best when exposed to a cold treatment of 2 C for 33 days and when stimulated by red and far-red irradiation. While the evidence is fairly substantial that monoecious tubers and turions require a chilling period to stimulate sprouting, the evidence for a chilling requirement for dioecious tubers is not as well supported.

Van and Steward (1990) reported that, *in situ*, monoecious tubers survived in the undisturbed sediment for a period of over 4 years in a study conducted in south Florida. It was postulated that persistence of monoecious tubers was regulated by an environmentally imposed enforced quiescence which prevent a rapid depletion of the tuber population through excessive germination *in situ*. Unfortunately, this is the only published study with the direct objective of dealing with tuber persistence and viability. Moreover, the longevity of monoecious tubers in more temperate areas of the United States where cold stratification is more likely has not been addressed. Harlan et al. (1985) reported that in three North Carolina lakes monoecious tubers began to sprout in late March when water temperatures reached 11 to 13 C and continued through August. These authors noted it was peculiar that tuber sprouting stopped in the field in late August though temperatures remained optimal. Subsequent laboratory studies suggested no seasonality existed (chilling was not required) as sprouting rates were 85 to 100%. Although no studies have dealt specifically with tuber persistence in dioecious populations, Langeland (1993) has reported persistence of large numbers of tubers

(300/m²) for up to 2 years following treatment with the herbicide DSM. Sutton (1996) reported that following measurement of tuber densities as high as 887/m² in the North New River Canal, Florida, 5 years of contact herbicide treatment and the introduction of grass carp removed vegetative growth, depleting the tuber bank within 3 to 4 years.

Van and Steward (1990) also investigated longevity of monoecious turions and reported that turions either germinated or died after 1 year. Differences in germination between tubers and turions was attributed to differences in environmental conditions and more extreme fluctuations near the sediment surface (where the turions are located) favoring breaking of quiescence and increased sprouting *in situ*.

Madsen and Owens (1998) have recently reported that biomass increase of hydrilla in Central Texas during the growing season from May through September resulted from growth of overwintering shoots and rootcrowns, not tubers. These authors reported that tuber sprouting generally occurs during August. This is the first report that suggests a distinct period of sprouting for dioecious hydrilla.

Determination of factors that either inhibit or promote tuber germination are important in developing new strategies for long-term maintenance control of hydrilla.

Research Needs

While a great deal of research has been conducted on hydrilla propagules, the data are often conflicting and several questions remain. In order to determine the best management alternatives, research on factors affecting *in situ* sprouting of hydrilla tubers, or systems which better simulate *in situ* conditions deserve attention.

To date, the vast majority of research on tuber sprouting has been conducted on tubers that have been removed from the sediment, thereby disturbing the propagules and exposing them to environmental factors (light, oxygen, reduced CO_2) that they do not experience in flooded hydrosoils. Sprouting of these propagules is often greater than 90% within a two week period, which strongly suggests an environmentally-imposed quiescence, as opposed to an innate dormancy. Furthermore, laboratory tests are often conducted for 1 to 2 weeks and conclusions that are drawn may be misinterpreted, also contributing to the accounts in the literature. For example, addition of exogenous growth regulators, or light may stimulate the rate of sprouting, but not necessarily the overall sprouting rate. In addition, application of exogenous growth regulators (especially ethylene) are known to have different effects in an aerobic versus an anaerobic environment (Jackson and Pearce 1991). The effect of exogenous hormone application on tuber sprouting in an anaerobic environment would better simulate the conditions in which tubers are found.

While suitable temperatures (13-35 C) are an absolute requirement for sprouting, it would not appear that temperatures in this range necessarily trigger *in situ* sprouting. In a study of 5 South Florida lakes, Sutton and Portier (1985) found no seasonal trend apparent for the sprouting of dioecious tubers or turions even though water temperatures were never below 15 C.

One phenomenon that has been observed by several authors (Mitra 1964, Haller et al. 1976, Van and Haller 1979, Joyce et al. 1992, Langeland 1993) is that dioecious tuber sprouting remains limited under a vegetative canopy, whereas rapid removal of the canopy by mechanical or chemical means greatly stimulates sprouting. Many hypotheses

have been proposed to explain this phenomenon, but none have been tested. Chemical and physical changes in the rooting medium such as changes in CO_2, increased oxygen, light penetration, and temperature changes have all been proposed.

Based on the previous scenarios that have been suggested to stimulate tuber sprouting, it can be inferred that the vertical position of the tuber within the substrate could play a significant role in its sprouting and survival potential. Data from natural plant populations of *Potamogeton* spp. and *Vallisneria americana* suggests non-uniformity in vertical distribution of propagules (Rybicki and Carter 1986, Spencer 1987, and Spencer and Ksander 1990). However, Rybicki and Carter (1986) showed that the majority of *Vallisneria* tubers were found at distinct depth intervals that differed based on sediment type. Harlan et al. (1985) found that monoecious hydrilla tubers in most samples collected in three North Carolina lakes were most dominant at depth intervals of 0 to 8 cm, however, up to 50% of the propagules could be found from 8 to 12 cm deep.

Depth distribution of hydrilla tubers may be particularly important in regards to the potential of light and oxygen to stimulate sprouting. Whereas previous studies have evaluated the effect of planting depth on propagule survival, no studies have evaluated if *in situ* sprouting is related to vertical position in the sediment. Moreover, sediment type may play a significant role in the sprouting of hydrilla tubers. Van and Haller (1979) demonstrated higher rates of sprouting following herbicide treatments of hydrilla growing in builders sand or gravel versus clay or organic soils. It was hypothesized that changes in gaseous constituents in the coarse hydrosoils may have played a key role.

The longevity of dioecious tubers *in situ* is currently unknown and would provide valuable information for plant managers. Evidence from field sampling following

fluridone treatments, management with grass carp, or a combination of these suggests a decrease in tuber populations over time, however, viable tubers can at least remain for several years posttreatment (Sutton 1996, A M Fox personal communication)

Although extensive research has been conducted on hydrilla tubers, our knowledge of the factors affecting their *in situ* longevity, quiescence, and sprouting remains inadequate While the Florida DEP objective is to bring hydrilla under maintenance control, the success or failure of this program will depend on the longevity, sprouting dynamics, and subsequent ability of sprouted tubers to become established.

A better understanding of tuber quiescence, factors that stimulate sprouting, and population dynamics would provide key information to support the direction of current maintenance control programs To accomplish this goal, the objectives of this research were (1) to determine the vertical distribution of tubers in the sediment and subsequent impacts of tuber position on *in situ* sprouting dynamics, (2) to test the hypothesis that removal of the vegetative canopy stimulates *in situ* sprouting of tubers, (3) to evaluate tuber sprouting and population dynamics at the field level following several control methods (4) to develop methods to improve laboratory evaluations of factors that impact sprouting of tubers once they are removed from the sediment, and (5) to conduct laboratory evaluations to focus on hormones and other compounds that likely inhibit sprouting of tubers

CHAPTER 2
VERTICAL DISTRIBUTION OF HYDRILLA TUBERS AND INFLUENCE ON SPROUTING AND ESTABLISHMENT

Introduction

Although many researchers have reported on total tuber density, few studies have focused on the subsequent sprouting of these propagules. Basic questions concerning how management techniques influence tuber sprouting and quiescence have received limited research attention. These questions are especially relevant when considering the length of time an aquatic system should be managed to prevent rapid recovery of the tuber bank. It remains unclear if tubers play a significant role in the rapid re-infestation of treatment sites due to stimulation of sprouting rates following various management techniques.

One of the major difficulties with field sampling of tuber populations to determine sprouting dynamics is the high degree of variability inherent when sampling tuber populations. Studies can be labor intensive, and due to high variability, may not yield data which accurately describes sprouting. Following several years of field sampling in four south Florida lakes, Sutton and Portier (1985) reported that tuber sprouting was generally random and non-seasonal. It should be noted that this research was conducted in areas that supported high densities of hydrilla for several years, and therefore sprouting response to management was not addressed. While the combination of high spatial

variability and random sprouting has discouraged field research in this area, recent studies by Sutton (1996) and Fox and Haller (personal communication) suggest that intense chemical management over 2 to 4 years (i.e., not allowing new tubers to be formed) can substantially deplete the tuber bank. Nonetheless, these studies have not determined sprouting rates and survival following treatment.

The sprouting and survival of aquatic propagules may be significantly impacted by their vertical distribution in the sediment (Titus and Hoover 1991). Although supporting data from aquatic systems is scarce, reports from terrestrial literature on several weedy species show that vertical position of seeds in the soil markedly influence whether or not seeds will germinate and survive (Buhler et al. 1997). While previous studies with dioecious hydrilla have focused on the total number of tubers within a given area of sediment sampled, only Harlan et al. (1985) have reported on the vertical distribution of monoecious tubers in 3 North Carolina lakes. These studies only quantified vertical distribution and no data was provided on how this distribution pattern affected sprouting rates or subsequent survival of the plants. Investigations on the depth distribution of vegetative propagules of submersed aquatic species other than hydrilla is also limited. This lack of information is unexpected because vegetative reproduction is known to be of great importance to survival and perrenation of submersed macrophytes (Sculthorpe 1967).

Bartley and Spence (1987) surveyed the literature and concluded that propagules of aquatic plants apparently do not display true dormancy and that there was a wide variation in which environmental factors were responsible for the release from dormancy. Nonetheless, based on the available literature, hydrilla appears to be somewhat unique

among submersed aquatic species in its ability to sustain a significant and viable population of quiescent vegetative propagules over a period of several years. For example, while species such as the exotic plant *Potamogeton crispus* L. annually produce turions, a very small percentage are thought to remain dormant for more than one season (Sastroutomo 1981). Although Sastroutomo (1982) suggests that aquatic propagules of several aquatic species can remain dormant, his classification system of short and long term dormancy does not provide unequivocal quantitative data. As many submersed species are generally viewed as beneficial to aquatic systems, there is generally a paucity of data concerning long-term propagule quiescence and response to major disturbances, especially management.

Following sprouting, the vertical distribution of aquatic propagules has been hypothesized to affect subsequent establishment and survival of some aquatic species. Rybicki and Carter (1986) reported that the majority of eelgrass *(Vallisneria americana)* tubers were distributed between 10 and 20 cm in silty clay and between 5 and 15 cm in sand. Subsequent laboratory studies demonstrated that survival of plants grown from tubers placed at different depth intervals decreased significantly with increasing sediment depth up to 30 cm. *Potamogeton gramineus* tubers have been found to be greatest at intervals between 6 and 10 cm of soil depth in two California irrigation canals (Spencer and Ksander 1990), whereas *Potamogeton pectinatus* tubers were found distributed throughout soil depths up to 23 cm in three California irrigation canals (Spencer 1987, Spencer and Ksander 1990). Placement of *Potamogeton pectinatus* and *Potamogeton gramineus* tubers at increasing depth intervals (up to 20 cm) resulted in decreased plant survival and vigor. Studies have also demonstrated that increased propagule size

was related to successful establishment following sprouting (Spencer 1987, Titus and Hoover 1991)

While most published studies have evaluated the influence of planting depth on survival and vigor of the plant, none of these studies was designed to determine how the effects of propagule depth distribution may affect the rates of sprouting. Observations in sediment cores have suggested that sprouting of *Potamogeton grammeus* was not influenced by their depth in the sediment. It is currently unknown if the position of a hydrilla tuber in the sediment influences sprouting.

Water temperatures between 15 and 35 C are generally thought to be a requirement for tuber sprouting (Haller et al. 1976, Steward and Van 1987), however, temperatures in this range do not necessarily stimulate a large proportion of the hydrilla tubers to sprout through the spring, summer and fall. In fact, in South Florida, sediment temperatures generally remain in this range throughout the year. As noted earlier, it has also been hypothesized that the position of the tuber in the sediment profile may be related to its ability to sprout due to exposure to changing environmental gradients. Tubers found at different sediment depths are likely to be exposed to different environmental variables such as light, oxygen (redox chemistry), and CO_2 levels (Titus and Hoover 1991). While light is known to stimulate tuber sprouting following their removal from the sediment, high levels of CO_2 have been shown to be inhibitory (Miller et al. 1976). Since light can only penetrate a few mm in terrestrial soils (Egley 1986, Benvenuti 1995), it is likely this value would be further reduced in aquatic sediments due to attenuation of light by water and the fact that the small particles that characterize sediments where plants grow prohibit light penetration to tubers.

Field Studies

Due to the potential importance of vertical distribution of hydrilla tubers in the field, several sites were sampled to determine the patterns of distribution in different aquatic systems. As noted above, there is not currently any published data that documents vertical distribution of dioecious hydrilla tubers or evaluated the effects of vertical distribution on potential for *in situ* sprouting.

Materials and Methods

Study 1. Vertical Distribution of Hydrilla Tubers Under Natural Conditions.

A 2-m drawdown (Nov 1995 - March 1996) at Rodman Reservoir in North Central Florida, allowed sampling of vertical tuber distribution in February and March 1996 at four locations known to support luxuriant hydrilla growth. Two sites located in an area characterized by sandy sediments were chosen based on water levels maintained at the reservoir. A site hereafter referred to as the lower sand site is under approximately 2 m of water at full pool, while a site referred to as the upper sand site is under approximately 1 m of water at full pool. Two sites were also chosen in areas characterized by highly organic muck sediments. The site referred to as the lower organic site is under approximately 2 m of water at full pool while the site called the upper organic site is under 1 m of water at full pool.

A 30 by 30 cm metal sampler with sidewalls and a back wall 2 cm in height was constructed and used to collect samples at 2-cm intervals to a depth of 28 cm. Prior to sampling, a small trench was cleared with a shovel and the metal sampler was then pushed horizontally into the sediment, and the contents (a 30 by 30 by 2 cm slice of

sediment) were placed in a fine-mesh screen for processing. Sediments were washed over a wire screen (3mm) and tubers at each depth were quantified and saved for subsequent sprouting tests. Twenty-five samples were collected at each sampling site.

Tubers collected at each sediment depth were sorted to size class (<150 mg, 150 to 300 and >300 mg) and placed in 8 cm diameter petri dishes containing 75 ml of distilled water. Sprouting rates were determined in the laboratory at 7, 14, 21, and 28 d under both light (14L: 10D) and dark conditions at a temperature of 24 ± 2 C. Twenty tubers were placed in each petri dish, and treatments were replicated 10 times. Tubers that remained quiescent past 28 d were saved for use in later studies (Chapter 5).

Sediments were also sampled for vertical tuber distribution at a drawdown site on Lake Kissimmee, FL, in June of 1996. Ten samples from each 2 cm depth to 26 cm were collected at two sandy sites using the same procedures as described for Redman reservoir.

In addition to use of the metal plate previously described, a more traditional 10 cm diameter stainless steel coring device (originally designed for deep ocean sediment sampling) similar to a PVC corer described by Sutton (1982) was used to collect intact sediment cores from a lake and research ponds that had not experienced drawdown conditions. The intact cores were subsequently sectioned into 2 cm intervals to determine vertical tuber distribution and percent sprouted vs. quiescent propagules. Fifty core samples were collected from Lake Tohopekaliga in April 1996. Eight research ponds located at the Center for Aquatic Plants, Gainesville, FL, that were 100% covered with hydrilla, were also sampled by collecting twenty cores from each pond (160 samples) in June 1996. In addition, six hydrilla-infested ponds at the Austin Carey Research Forest

near Gainesville FL, were also sampled in May 1996 prior to employing management methods to control hydrilla A total of fifty samples was collected from each pond (300 samples) to determine vertical position of hydrilla tubers

Sediments for all sites (with the exception of the Lake Kissimmee site) were analyzed for % organic matter, % moisture content, and sediment density through the vertical profile. Six samples from each site were sectioned into 2 cm intervals (up to 18 cm) and each section was analyzed separately For determination of percent organic matter, a 5-g sediment sample was collected and weighed to the nearest 0 001 g in a pre-weighed crucible Samples were then placed in a muffle furnace at 550 C For moisture content and density, fresh sediments were weighed upon removal from the water, placed in crucibles of a known volume (20 mL), and placed in a drying oven at 70 C for 120 hr

Differences in depth distribution of tubers at each sampling site was determined by ANOVA. If differences were detected, mean values were separated by an $LSD_{0.05}$

Study 2. Determination of *in situ* Sprouting.

In order to determine *in situ* sprouting depths of tubers following a drawdown, 10 cm diameter by 30 cm length pvc pipes were driven into the sediment and intact cores were extracted from sites 1-4 (high and low sand and organic sites) at Rodman Reservoir in February 1996 The intact core samples were immediately transported to the Center for Aquatic Plants (CAP), Gainesville, FL, and placed in three 900 L concrete vaults. A 20 cm layer of commerical potting soil was placed in each vault to seal the core bottoms and maintain reducing conditions at the bottom of the vault A total of 240 cores were collected (60 per site) and placed in the vaults and then flooded. Sediment temperature and redox potential were monitored in representative cores on a weekly basis.

Temperature was measured by inserting a thermocouple probe into the sediment at 3, 6, 12, and 15 cm To determine sediment redox potential platinum electrodes, specially constructed for field use, were inserted into the cores at 3, 6, 9, 12, 15, 18, and 21 cm Methods for redox determination were similar those described by Faulkner et al. (1989). Redox readings were taken using a hand-held pH/mV meter with a calomel reference electrode and adjusted to the hydrogen electrode standard by adding 224 mV Sediment pH was determined in June, August, and October by taking the average reading of five cores that were collected and sectioned into 2-cm intervals

Fifteen cores from each sample site were harvested from the CAP vaults on June 6 (4 months), August 6 (6 months), and October 15 (8 months), 1996 Sediment was extruded from the bottom of the pvc cores and sectioned into 2 cm intervals. Tubers were quantified as being quiescent, rotted, or sprouted. By the October sampling period some tubers were identified that had sprouted (a hole was observed where the tubers had previously been attached to a shoot apex yet they remained turgid and were obviously not rotting) but no longer remained attached to a growing shoot. These tubers were still classified as sprouted along with the tubers that maintained an attachment to the sprouted plant

Following the three harvests in 1996, fifteen cores remained from each sampling site following the October harvest At this time (October 1996) all vegetative biomass was removed from the cores from the lower sand and upper organic sites to prevent tuber production through the fall, winter, and spring In contrast, cores labeled upper sand and lower organic remained vegetated and were allowed to form tubers through May 1997 On May 4, 1997, cores were harvested and tuber values and sprouting were quantified

In addition to cores collected at Rodman Reservoir during the drawdown, 100 intact core samples were collected from untreated hydrilla infested ponds at the Austin Cary forest near Gainesville, FL in late April 1997. Fifty cores were immediately placed in a 900 liter concrete vault that contained a 20 cm layer of potting soil on the bottom to maintain reduced conditions. In the remaining 50 cores the top 3 cm of sediment was extruded and removed and the cores then placed in the 900 l vaults as described above. Manipulating the position of the tubers in the vertical profile (removing the top 3 cm of sediment) was done to determine if this would impact subsequent sprouting rates compared to undisturbed cores. In addition, this manipulation resulted in the removal of all vegetative biomass from these cores. Based on prior sampling (over 200 samples), it had been determined that the majority of tubers were located between 4 and 6 cm in the Austin Cary sediment profile. Redox potential at 3 and 6 cm and sediment temperature (6 cm) were monitored as described for the Rodman cores every 30 days through the end of the study. Fifteen cores from each treatment were harvested on June 20 (70 d) and September 18, 1997 (159 d), to determine percent sprouting, quiescence, and rotting.

The percent of tubers sprouting at each depth were compared by ANOVA at each sampling date. Following harvests, quiescent tubers were evaluated for % viability in petri dishes over a 21-d period.

Results and Discussion

Study 1. Vertical Distribution in the Field

Observations during sampling at Rodman Reservoir suggested that the sediments from the organic sites remained saturated (trenches continued to fill with water during

sampling), whereas the sediments in the sandy sites were well drained. Subsequent redox readings taken at 3, 6, and 12 cm from cores extracted from these sites indicated that redox in the sandy sites was characterized by slightly reduced sediments (+160 to +330 mV), whereas organic sites remained anoxic and highly reduced (-34 mV to -331 mV)

In the sandy sediments of Rodman reservoir, the vertical distribution of tubers was well-defined with maximal values between 8 and 10 cm, followed by a marked decline in tubers at 12 cm (Figure 2.1) It is unknown if the comparative scarcity of tubers from 0 to 6 cm is due to lack of production at these depths or related to enhanced sprouting rates which deplete the tuber bank more readily than at deeper positions. Data from the 9-12 cm deep zone showed several changes in % moisture, organic matter, and bulk density Apparently these or other unknown parameters stimulated maximum tuber formation at the 8 to 10 cm depth. However, previous studies by Barko and Smart (1986) showed that increased sediment density impacted hydrilla rooting depth and Coley and Kay (1994) have suggested that tuber depth distribution may be predicted by using a soil penetrometer to measure hydrosoil compaction.

In contrast to the sandy sediment study sites in Rodman reservoir, tubers in the organic sediments were more randomly distributed between depths of 4 to 18 cm (Figure 2 1) Tubers found at greater depths in the organic sediment compared to the sand sediments suggest that increased rhizome penetration may occur due to the lower density of these sediments at depths up to 18 cm (Table 2 1) The organic sediments were quite heterogenous and contained many large debris (sticks, rhizomes, twigs)

Tuber sprouting rates in the lab were in the range of 65-96% and analysis of variance indicated that sprouting rates were independent of tuber depth in the sediment or

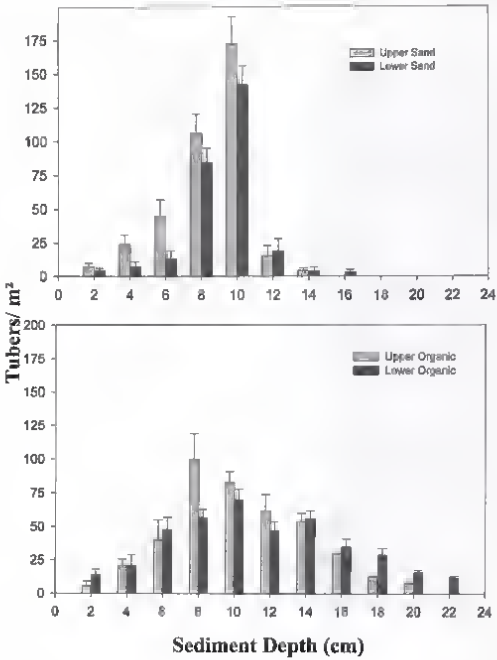

Figure 2 1 Depth distribution of hydrilla tubers collected at Rodman Reservoir during a drawdown. Each bar (+ 1 SD) represents the average number of tubers/m² at each depth interval (n=25)

Table 2 1 Sediment characteristics and distribution of hydrilla tubers in PVC cores collected at Rodman Reservoir Samples were processed immediately following removal of the flooded cores from concrete vaults

Sample Site (Depth cm)	moisture %	organic matter %	density g / cc	Total % of Tubers*
Upper sand				
3cm	52	6.4	0 74	2
6	47	5 2	0 83	8
9	34	1.3	0 95	36
12	20	0.6	1.20	49
15	16	0 6	1.33	4
18	15	0 6	1 41	0 5
Lower sand				
3cm	56	7.4	0 70	0 5
6	46	5 1	0 87	6
9	40	3 2	0 98	29
12	21	0.5	1 25	55
15	17	1.3	1 45	5
18	16	1 5	1 51	0 5
Upper organic				
3cm	66	17 1	0 44	1
6	49	21.3	0 67	14
9	44	19 5	0 88	26
12	39	6 8	0 94	33
15	27	5 1	0 97	12
18	27	2 8	1 18	12
Lower organic				
3cm	85	32 5	0 39	2
6	90	56 1	0 56	17
9	77	27 2	0 71	15
12	47	9 7	0 80	29
15	35	7 1	0 87	14
18	36	7 9	0 99	13

*Tuber values are reported at a distinct depth interval however these values represent the total number of tubers found in a 3 cm sediment sample (i.e 0-3 cm 3-6 cm)

tuber size within each sampling site. Therefore data collected for depth and tuber size from each sampling site were combined to provide a single value for percent sprouting, rotting, and quiescence. Sprouting rates for tubers maintained in dark conditions were delayed compared to tubers exposed to light conditions, however, by 28 d, sprouting in the dark exceeded 80% and was generally not different from values obtained in the light (Table 2.2). Allowing these sprouting tests to run for longer periods of time indicated that while light increased sprouting rates it was not a requirement for sprouting.

Tubers collected from sandy sites were more predisposed to rotting in the sprouting tests and therefore percent sprouting in the sandy sediments was reduced compared to organic sediments. During field collection it was also noted that a much higher percentage of rotten or rotting tubers were found in the sandy sediments (19%) compared to the organic sediments (2%). Redox probes placed in PVC cores collected from the four sampling sites indicated that the sandy sediments were often characterized by oxidized conditions to a depth of 12-14 cm, whereas, the organic sediments remained reduced throughout their entire depth. It is speculated that increased aeration of the sandy sediments in comparison to the organic sediments may have played a role in the significant increase in tuber decay observed in the sandy sites. This increased rotting in the sandy sediments during drawdown conditions was also noted by Haller et al. (1983). Introduction of oxygen into the sandy sediments may increase the potential for attack by aerobic fungal pathogens as has been described by Bernhardt and Dunnway (1986).

Tuber distribution in Lakes Kissimmee and Tohopekaliga was similar to that found in the sandy sites in Redman reservoir with a distinct maximum value followed by a sharp decline (Figure 2.2). Characterization of sediments from Lake Kissimmee also

Table 2.2 Percentage of hydrilla tubers sprouting and decomposing under light and dark conditions following collection from Rodman Reservoir under drawdown conditions in February 1996

	Light				Dark			
Sample Site	7	14	21	28	7	14	21	28
Upper sand								
Sprouting	44*	62	62	69	15*	43*	63	65
Rotting	23	31	31	31	15	20	25	25
Quiescent	33	7	7	0	70	37	12	10
Lower sand								
Sprouting	55	73	81	85	25*	47*	65*	83
Rotting	10	15	15	15	15	17	17	17
Quiescent	35	12	4	0	60	36	18	0
Upper organic								
Sprouting	67	87	96	93	37*	55*	72*	86
Rotting	4	4	4	4	5	9	9	9
Quiescent	29	9	0	0	58	36	19	5
Lower organic								
Sprouting	70	77	89	93	40*	61*	77*	88
Rotting	0	7	7	7	5	5	5	5
Quiescent	30	16	4	0	55	34	18	7

*significant differences between sprouting percentages of light and dark-exposed tubers at given sample date according to t test$_{0.10}$

Each value represents the average of 20 replicate samples, each containing ten tubers

indicated that below 12 cm, sediment bulk density increased to greater than 1 2 g/cc with a concomitant decrease in the % moisture content at these depths (Table 2.3)

Tuber distribution in ponds at the Center for Aquatic Plants that had been infested with hydrilla for 2 years also showed that tubers reached a distinct maximum value followed by a sharp decline as depths increased (Figure 2 2) Again, these were sandy sediments characterized by an increase in bulk density and decreased % moisture at sediment depths below where the maximum value of tubers was found (Table 2.3) A similar trend in tuber distribution was also found in ponds at the Austin Cary Forest; however tubers numbers generally peaked at shallower depths than observed in other sites (Figure 2.2) The Austin Cary site has supported a hydrilla population since 1974 and while there was some variability noted in the substrates within and between ponds depth distribution remained quite consistent.

Following collection of tubers from these sites, tuber sprouting rates in the lab were in the range of 85-100% and were independent of depth from which tubers were collected in the sediment or tuber size Tubers maintained in dark conditions sprouted later than light exposed tubers, however no differences in the total percent sprouting was detected by 28 d (Table 2 4) In contrast to the sandy sediments in Rodman, very few tubers rotted in these trials where drawdowns were not conducted, further suggesting that conditions created during drawdowns may favor decomposition of the tubers.

Although Harlan et al 1985 determined the vertical distribution of monoecious hydrilla tubers, the scale used was too broad (0-0 8, 0 9-8, and 8-16 cm) to show if the tubers were concentrated at a certain depth This data suggests that the depth of tuber formation may be related to sediment density and in many sites the majority of tubers

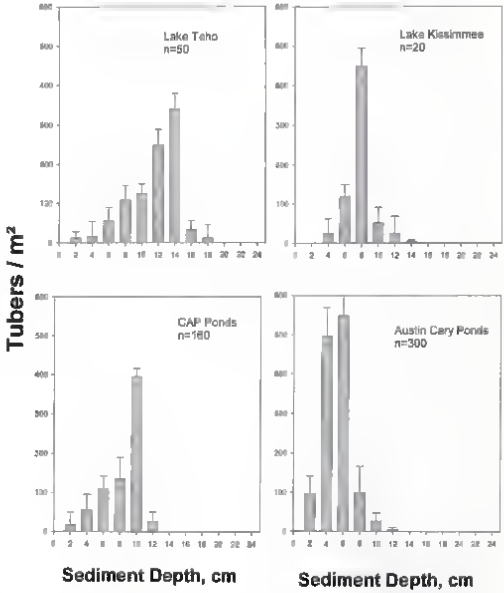

Figure 2 2 Depth distribution of hydrilla tubers collected at 4 sites in Florida. Data for Lake Kissimmee was collected during a drawdown with a large metal sampler (30 x 30 x 2 cm) whereas samples at the other sites were collected with a 10 cm diameter coring device Each bar (+ 1 SD) represents the average number of tubers at each depth interval

Table 2 3 Sediment characteristics and tuber distribution in cores taken from Lake Kissimmee, and research ponds at the CAP and Austin Cary Forest.

Sample Site (depth cm)	% moisture	% organic matter	density g / cc	Total % of Tubers
Lake Kissimmee				
3cm	46	11.4	0.85	0*
6	40	7 2	0 83	21
9	25	2 5	1.07	66
12	22	1 8	1.23	12
15	19	3 5	1 21	0.5
18	19	3 8	1.27	0
CAP Ponds				
3cm	53	8 2	0 81	3
6	33	2 1	0 91	21
9	24	1 9	1 09	19
12	20	3 1	1 25	54
15	19	2.5	1 20	4
18	21	1 8	1 23	0
Austin Cary Ponds				
3cm	43	4.3	0.91	8
6	21	1.8	1 13	81
9	16	1.2	1.20	7
12	18	2.2	1 25	3
15	15	1 8	1 21	0 3
18	17	2 1	1 19	0

*Tuber values are reported at a distinct depth interval, however these values represent the total number of tubers found in a 3 cm sediment sample (i e , 0-3 cm, 3-6 cm)

Table 2.4. Percentage of hydrilla tubers sprouting and decomposing under light and dark conditions following collection from Lakes Kissimmee and Tohopekiliga, FL, in March of 1996 and from replicated ponds at the Center for Aquatic Plants (CAP) and Austin Cary Forest, FL.

Sample Site	Light			Dark		
	7	14	28	7	14	28
Lake Kissimmee						
Sprouting	72	85	90	45*	70*	94
Rotting	0	0	0	0	0	0
Quiescent	28	15	10	55	30	6
Lake Tohopekiliga						
Sprouting	73	79	95	37*	64*	90
Rotting	0	0	2	4	4	4
Quiescent	27	21	3	59	32	6
Center for Aquatic Plants						
Sprouting	83	87	94	55*	71*	91
Rotting	0	0	0	2	2	2
Quiescent	17	13	6	43	27	7
Austin Cary Forest						
Sprouting	85	89	93	59*	77*	95*
Rotting	3	7	7	5	5	5
Quiescent	12	4	0	36	18	0

Significant differences between sprouting percentages of light and dark-exposed tubers at given sample date according to t-test$_{0.05}$

Each value represents the average of 20 replicate samples each containing 10 tubers

are located at a fairly specific depth in a given sediment. Redox potential was not correlated with depth of tuber formation as had been hypothesized. Although sediment density was correlated with vertical tuber distribution in sandy soils, it appears that tubers in highly organic soils are likely to be more randomly distributed in the sediment.

Determination of *in situ* Sprouting in Sediment Cores

Regrowth of hydrilla in the PVC cores collected from Rodman Reservoir was noted as the temperatures warmed to >18 C by Mid April, however this regrowth came from stem fragments buried in the organic sediments, and axillary turions near the sediment surface in both the sandy and organic sediments. Harvest of sediment cores in May showed that only a small fraction of tubers were sprouting in the sand and organic cores at any depths (Figure 2 3). Temperature and sediment redox data suggested that sand and organic sites had become similar following re-flooding of the cores (Table 2 5). Prior to any tubers reaching the sediment surface in May, hydrilla biomass had already increased dramatically from 0 to 4.2 ± 3 1 g dry weight/ core. Had the source of this early regrowth not been determined, it would have been easy to assume that recovery was occurring from sprouting tubers. The recovery observed in a two-month period (April May) from buried stem fragments and axillary turions resulted in near complete coverage of the 900-L concrete vaults by June.

The organic and sand sediments resulted in distinct differences in tuber sprouting following submersion of the cores. While the majority of tubers generally sprouted or rotted in the sand sediments, most tubers remained quiescent in the organic sediments throughout the sampling period (Figure 2 3). Over 85% of the quiescent tubers collected from the organic sediments in May, August, and October sprouted in petri

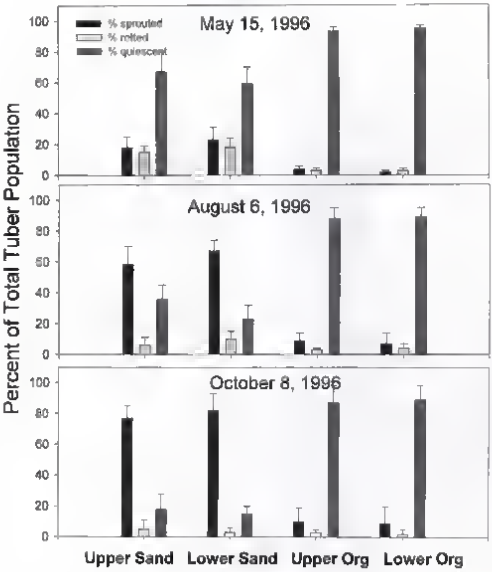

Figure 2 3 Hydrilla tuber sprouting, rotting, and quiescence recorded following the re-flooding of intact pvc cores collected at Rodman Reservoir in March 1996 Data collected for each date and site represents the average of 15 samples (+ 1 SD)

Table 2 5 Sediment temperature and redox profiles in PVC cores collected at Rodman Reservoir in February 1996

Sample Date	Upper sand	Lower sand	Upper organic	Lower organic
		Temp C (8 cm)		
3/11	18 4 ± 0 6	18 1 ± 0 6	18 0 ± 0 7	18 1 ± 0 4
4/1	20 9 ± 0 5	20 7 ± 0 4	20 4 ± 0 5	20 5 ± 0 6
4/15	22.5 ± 0 7	22 4 ± 0.3	22.3 ± 0 4	22 5 ± 0 6
5/15	25 7 ± 0 7	25 7 ± 0 5	25.3 ± 0 4	26 0 ± 0 8
6/15	26 8 ± 0 5	26 4 ± 0 7	27 0 ± 0 6	26 2 ± 0 3
7/15	25.2 ± 0 6	25 4 ± 0 6	25 1 ± 0 5	25 1 ± 0 5
8/15	25.5 ± 0 4	25 9 ± 0 6	25 1 ± 0 6	25 4 ± 0 6
9/15	26 1 ± 0 6	25.8 ± 0.3	26 4 ± 0 4	26 1 ± 0 4
10/15	23 5 ± 0 7	23 6 ± 0 6	23.2 ± 0 5	23 5 ± 0 7
		Redox mV (4 cm)		
4/1	-300	-318	340	329
4/15	-298	-320	-356	334
5/15	-319	324	345	325
6/15	-299	316	-330	315
7/15	-329	321	295	319
8/15	312	318	345	-334
9/15	285	319	-332	-325
10/15	-309	-321	-325	-319
		Redox mV (18 cm)		
4/1	-300	296	295	-291
4/15	-305	-303	-287	313
5/15	313	-310	-320	-331
6/15	-315	-304	-317	-316
7/15	316	305	-306	-314
8/15	319	311	294	-313
9/15	-319	299	311	-328
10/15	-313	-304	309	-319
		Redox mV (36 cm)		
3/15	-257	244	285	-305
4/15	-246	247	-270	-313
5/15	-229	246	269	-317
6/15	-240	233	-271	-316
7/15	245	247	274	-318
8/15	-236	-237	-274	-307
9/15	238	-234	281	-309
10/15	244	239	-289	-312

dishes within 10 d, once again suggesting that the observed *in situ* quiescence was environmentally imposed. Because no differences were detected in the percentage of tubers sprouting at a given vertical depth or sample time (data not shown) in the sandy sediments data is presented as the total number of tubers sprouting per core. Enhanced sprouting of tubers following a drawdown in sandy sediments versus organic sediments has also been noted by Heller et al (1983)

Harvest of the remaining cores one year later in May 1997 showed that tuber populations were replenished in the upper sand sediments and had increased by 67% over the initial levels in the lower organic sediments (Figure 2 4) The lower sand and upper organic cores had vegetative cover removed in the fall of 1996 (and monthly thereafter), and were not allowed to form new tubers Tuber populations in the lower sand sediment were significantly depleted, whereas the upper organic sediments sustained a relatively stable population of tubers Differences between the organic and sand sites were attributed to tubers remaining quiescent in the organic site compared to the high degree of sprouting and rotting that occurred in the sandy sites throughout the previous summer

Results suggest that in some situations, a drawdown may be a highly effective tool for stimulating tuber sprouting and thus offer a tool for developing hypotheses on conditions favoring sprouting Nonetheless, as noted in Rodman reservoir, the heterogeneity of sediment types would have resulted in a significant reduction in the tuber bank in the sandy areas, and little impact in areas containing organic sediments A subsequent visit to Rodman reservoir in April of 1997 indicated that hydrilla had reinfested both sampling areas either as a result of tuber sprouting or more likely growth of vegetative fragments. While drawdowns alone for hydrilla control were shown to be

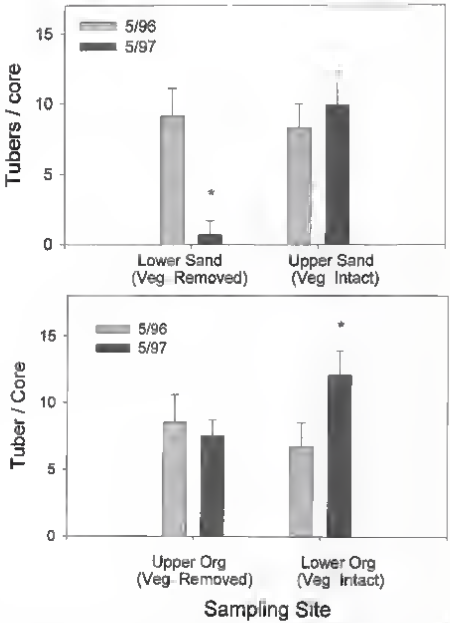

Figure 2.4 Hydrilla tuber density in pvc cores containing either a sand or organic sediment collected from Rodman Reservoir in March 1996. Pretreatment density measured in 5/96 was followed by either allowing hydrilla to recover (Veg. intact) or herbicide treatment of the vegetation (Veg. removed) to prevent tuber production. Each bar (+ 1 SD) represents the mean of 15 samples. Asterisks above the bars indicate differences in tuber populations between 1996 and1997 according to a t-test 0 05

ineffective in the late 1970's and early 1980's (Miller et al 1976, Haller et al 1983), a drawdown followed by control techniques to prevent hydrilla re-establishment and subsequent tuber production may be a viable management technique, particularly in sites with sand-dominated sediments.

The length of time required for a drawdown to stimulate tuber sprouting remains unknown, but based on differences observed in this study between sandy and organic sediments, time requirements are likely related to sediment type and the degree of sediment oxidation that occurs. It is currently unknown if only short term exposure to oxidized conditions is necessary to stimulate *in situ* sprouting or if these effects occur over longer periods of time

Physical Manipulation of Sediment Cores did not Stimulate Sprouting

Tubers in sediment cores collected from Austin Cary ponds had low sprouting rates throughout the short-term study. No differences in sprouting rates were detected for manipulated and undisturbed cores (Table 2 6) Redox readings at 3 and 6 cm sediment depths suggest that manipulating the sediments also had no impact on sediment redox properties Some variation in sediment temperature was noted, but this was likely due to differences in the amount of vegetative cover between manipulated and undisturbed cores The sprouting rates observed in the cores were consistent with those observed *in situ* in the ponds based on extensive core sampling (Chapter 4) Nonetheless, greater than 90% of these quiescent tubers sprouted within 10 d when removed from the cores The fact that tuber sprouting did not increase in manipulated cores tends to support a hypothesis which suggests that sprouting is not related to vertical position of the tuber in the sediment. It should be noted that the similarities between redox readings in

Table 2 6. Comparison of tuber sprouting and sediment redox in undisturbed pvc cores collected at the Austin Cary Forest with cores in which the top 3 cm of sediment (including vegetative biomass) was removed

	Percent Tuber Sprouting	
Treatment	June 1997	Sept 1997
Intact Core	8 ± 4	10 ± 4
Top 3 cm removed	11 ± 6	13 ± 3

	Sediment Redox Potential (mv)[a]			
Treatment	May 1997	June 1997	Aug 1997	Sept 1997
Intact Core				
3 cm	-267	-289	-245	299
6 cm	-299	-244	-219	277
Top 3 cm Removed				
3 cm	-233	-218	-287	248
6 cm	-271	222	231	265

[a]Each value represents the mean of 15 pvc cores
[b]Each value represents the mean of readings from 4 redox probes.

manipulated and undisturbed cores suggests that the tubers did not experience any redox chemistry changes Therefore, outside of small sediment temperature variations (due to vegetation removal) and the improbable chance of increased light penetration into the sediment (highly unlikely at depths exceeding 2-3 mm) there were likely few difference between the treatments. Although there has been some suggestion that tubers are stimulated to sprout following scouring of sediments in some reservoir systems, outside of physically unearthing the tuber, these results suggest that sediment scouring would not increase tuber sprouting

Mesocosm Studies

Observations made in the previously described field studies led to a hypothesis that the depth of tuber formation is largely affected by mechanical impedance This limitation in depth of formation has been described for agronomic crops such as potato and peanuts (Ewing and Struik 1992) and it has been suggested that when stolon tips push against soil particles, their extension growth is restricted due to a release of ethylene and tuberization is favored (Vreugdenhil and Struik 1989) As noted in the previous studies, the interest in the depth of tuber formation relates to how position in the vertical profile impacts the potential for sprouting and establishment. To address these questions, studies were conducted to determine if depth of tuber formation is affected by mechanical impedance. Furthermore, if a technique can be developed to grow tubers at specific depths, conclusive studies could be conducted to determine if tuber location in the sediment profile affects subsequent rates of sprouting and survival Results obtained with cores collected from the field were somewhat equivocal due to the fact that much of

the work was conducted with sediments that had experienced a long-term drawdown. Moreover, major differences in sprouting were found between the sandy and organic cores

Materials and Methods

In order to determine factors affecting depth of tuber formation and subsequent sprouting rates, a series of mesocosm studies were conducted. PVC cores (10 cm diameter and 30 cm depth) were marked at 3 cm intervals to 30 cm. Cores were filled with a selected soil type and Styrofoam barriers (1.5 cm thick) were placed at soil depths of 3, 6, 9, 12, 15, 18, 21, 24, and 27 cm. The Styrofoam barriers were used to allow root penetration, nutrient flux, and exchange of gaseous constituents of the sediment, while not allowing rhizome penetration. Five hydrilla apices, 10-15 cm in length, were planted in each pvc core. Each treatment was replicated 6 times and cores were placed in 900 L vaults (210 cm long x 70 cm wide x 94 cm deep) in a completely randomized design.

Study 3 - Impact of Mechanical Impedance on Depth of Tuber Formation

This study was initiated in a growth room on July 23 1996 at the Center for Aquatic Plants. Six PVC cores were filled with either nutrient amended (15 g of Osmocote 20-10-10 per Kg) builders sand, or a commercially sold organic peat consisting of 95% organic matter. Cores containing hydrilla apices were randomly placed in a 900 L fiberglass vault and water temperature was maintained at 24 ± 2 C for the duration of the study. Overhead lighting was provided by 250 W Gro-Lux bulbs and light intensity at the water surface was measured at 420 ± 20 µmol/m²/sec. To allow hydrilla to become established the photoperiod was set to 16L: 8D for 28 days. Following canopy formation,

the photoperiod was adjusted to 11L:13D for the remainder of the study. Redox potential was measured in each sediment at 3 cm intervals on a weekly basis and sediment pH was measured 2 weeks into the study and at the final harvest. The final harvest was conducted on November 14, 1996. Shoot biomass was harvested and dried to a constant weight (70 C for 48 hr) and cores were extruded and sectioned into 3-cm intervals to determine tuber distribution.

Study 4

Study 4 was initiated on September 3, 1996 at the Fifvens Arm research site in Gainesville, FL. Six replicate cores were filled with either nutrient amended (1.9 g of Osmocote 20-10-10 per Kg) builders sand, commercial organic peat (95% organic matter), or a loam commercial potting soil (Vitahume®) that has been used for numerous hydrilla mesocosm studies in the past. Styrofoam barriers were placed in the cores at 3 cm intervals through 27 cm. Cores containing five meristems of hydrilla were placed in 900 L concrete vaults and exposed to ambient outdoor conditions. The hydrilla had formed a thick surface mat by October 3, 1996. Sediment redox at 3 cm intervals was measured on a monthly basis. The final harvest was conducted on May 15, 1997. Shoot biomass in each core was harvested and dried to a constant weight (70 C for 48 hr) and sediment was extruded and sectioned into 3-cm intervals to determine tuber quantities.

An exact duplicate of this study was set up as described in the preceding paragraph. However, in contrast to the final harvest described above, the vegetative canopy was chipped at the sediment surface on May 15, 1997, but the cores were allowed to remain intact. Following removal of the canopy, a chelated copper (ethylenediamine complex) was applied to the vault at a rate of 2.0 mg/L to remove all remaining shoot

biomass. Three cores were harvested at 60 and 120 days following removal of vegetative biomass, and sectioned into 3 cm intervals to determine the percentage and depth of tuber sprouting in each treatment. In contrast to earlier studies in which cores were collected in the field, all of the tubers in these studies were of a known age

Study 5

Study 5 was initiated on January 19, 1997 at the CAP. Six replicate PVC cores were filled with either a natural organic sediment (40 % organic matter) collected from Orange Lake, FL or the commercial organic peat collected and saved from Study 1. Organic peat saved from the first study was used based on the assumption that any potential toxic leachates would be reduced and this may lead to a reduction in tuber mortality observed in Study 3. The soft organic sediment collected from Orange Lake was separated into a set that had been passed through a 3 mm screen to remove any large particulate materials, and a set that received no screening. Styrofoam barriers were placed at 6, 12, 18, and 24 cm. Cores containing five hydrilla apices were placed in a 900 L concrete vault in a completely randomized design. An immersion heater (Model M Electric S, at 1.0 kW) with a thermostat was placed in the vault to raise ambient water temperatures to promote growth and tuber production. Cores were harvested on May 18, 1997 and shoot biomass and tuber distribution were determined as described above.

An exact duplicate of this study was set up as described in the preceding paragraph. However, in contrast to the final harvest described above, the vegetative canopy was clipped at the sediment surface on May 18, 1997 and the cores were then treated with endothall at a rate of 5 mg/L to remove any remaining above ground biomass. Three cores from each treatment were harvested at 60 and 120 days following

removal of vegetative biomass, and sectioned into 3 cm intervals to determine the percentage and depth of tuber sprouting. All of the tubers in these studies were produced during a 4 month period and were of a known age

Data Analysis

Depth of tuber formation data and sprouting data following management were subjected to ANOVA, and if significant differences in tuber production or sprouting were noted at different depths, means were separated by an $LSD_{0.05}$. Due to differences in protocols (i.e., sediment types, filtering procedures) data was analyzed separately for each study

Study 6. Impacts of Sediment Manipulation on Tuber Sprouting

In September of 1996, thirty PVC cores were filled with fertilized potting soil and a Styrofoam barrier was placed 9 cm deep in each core. Four apices of hydrilla were established in each core and then cores were placed in 900 L concrete vaults filled with well water. Plants were allowed to grow and form tubers through May 1997. At this time, ten cores were removed from the water and sediment was extruded through the top of the core until the top 6 cm of sediment had been removed, also removing the vegetation. Following this manipulation, the remaining tubers were covered by a 1-3 cm layer of sediment. An additional ten cores were removed and the sediment was extruded through the top of the core until tubers embedded in the Styrofoam barrier (approximately 12-19 tubers) were partially exposed. Care was taken to allow a thin layer (1-5 mm) of sediment to remain in contact with the tubers. The remaining ten cores were left intact with extensive vegetative cover. All cores were placed back in the vault in a completely randomized design and given a 90 day period prior to harvest to determine tuber

sprouting percentages Redox potential was measured at 4 d, 31 d, and 60 d into the study and readings were taken just above the Styrofoam barriers

This study was repeated in January 1997 An emersion heater was placed in the venlt to bring water temperatures 6-10 C above ambient temperatures. Previous studies indicated that simply increasing ambient water temperatures in the winter greatly improved hydrilla growth and tuber production Cores were collected in May 1997 treated as described above, and harvested at 90 d to determine tuber sprouting rates

Data were subjected to ANOVA, and if differences were detected, means were separated by an $LSD_{0.05}$ Analysis indicated differences existed between the studies, and therefore data were analyzed separately for each study

Study 7. Hydrilla Growth and Tuber Production. Impact of Sediment pH

In studies 3 and 4 described above, hydrilla grew vigorously on organic peat sediments and formed numerous rhizomes in response to the short photoperiod However, during harvest of the previous studies it was noted that these rhizomes grew approximately 3-6 cm into the sediment and then decayed The impact of increasing organic matter on the vegetative growth of hydrilla remains equivocal, with reports of both significant decreases in biomass (Barko and Smart 1983), as well as reported increased biomass (Spencer et al 1992) Nonetheless, the complete lack of tuber production following rhizome initiation noted in studies 3 and 4 has not been reported The low pH measured in the peat sediments (range of 4 6 to 5 8) was thought to be related to the lack of tuber production. Spencer and Ksander (1995) have demonstrated that treatment of hydrilla tubers with acetic acid greatly increases mortality, which presumably could be due to a decrease in sediment pH Studies were conducted to

determine if the lack of tuber production in the peat sediments could be ameliorated by a pH adjustment or if a more complicated mechanism such as leachates or metal toxicity from the peat was involved.

Commercial peat used for previously described studies, as well as peat that had been used in the previous studies, was amended with lime (calcium carboonate) at rates of 0, 1, 2.5, and 5% by air dry soil weight. The lime was used to increase sediment pH and was thoroughly mixed through the peat sediment prior to planting hydrilla. In December of 1996, amended sediments were placed into 96, 1 L plastic pots and 4 apices of hydrilla were planted. Pots were placed in a 900 L concrete tank (containing an immersion heater) in a completely randomized design. Sediment pH was measured by temporarily removing pots from the sediment and placing a pH probe into the sediment and taking the average of three readings. Redox probes were placed into three pots of each treatment rate 72 hr prior to harvest and redox potential was measured just prior to harvest. Each treatment rate was replicated six times, pots were harvested in May, 1997, and shoot biomass and tuber production was measured for each treatment. This study was repeated in November 1997, and pots were harvested in May, 1998.

Shoot biomass and tuber production were subjected to analysis of variance. If treatment differences were detected, means were separated by an $LSD_{.05}$. Differences were not noted between studies, therefore data from studies 1 and 2 were pooled.

Study 5. Influence of Depth in the Sediment Profile on Tuber Emergence

Due to the erratic nature of *in situ* tuber sprouting from cores collected in the field, determination of the influence of depth on the ability of the sprouting tuber to reach the sediment surface was difficult to ascertain. Therefore, in order to determine the

effects of depth of the tuber in the sediment on its ability to emerge and become established was also tested. Plexiglass chambers (46 cm long x 30 cm tall x 1.2 cm thick) were constructed to allow visual assessment of sprouting and elongation during the course of the study. Face plates were removed from the plexiglass chambers and sediment was poured to fill the chamber space. Chambers were filled with either a commercial potting soil previously used for hydrilla studies, builders sand, or an organic sediment collected from Orange Lake, FL. A cm ruler was then placed at a 45 degree angle diagonally across the chamber. A single tuber was placed at each 2 cm interval on the 45 degree angle, therefore each 2 cm diagonal interval was equal to a 1 cm increase in vertical depth. The tubers used for this study had been removed from the sediment for 24 hours prior to planting. Previous studies had shown that this treatment would increase rates of sprouting. In addition, tubers were sorted into size classes of those between 100 and 200 mg and those between 350 and 450 mg. Face plates were tightly secured and the chambers were submerged in concrete vaults and sunk in the sediment (30 cm deep flooded potting soil) to exclude light penetration through the sides of the plexiglass. Chambers were removed every two weeks and elongating tubers that had emerged to the sediment surface were counted. Each treatment was replicated 4 times. Studies were initiated in June of 1996 and April of 1997 and run for 4 months. Studies conducted in April 1997 used filtered sediments from the Austin Cary ponds (high sand fraction) in place of builders sand. The objective of these studies was to determine the influence of depth in the vertical profile on the ability of the tuber to emerge to the sediment surface. Due to the optimal growth conditions in the mesocosm vaults (high light penetration) no attempts were made to compare the ability of tubers to establish once they emerged

Main effects impacting tuber emergence included sediment type (3), depth profile (7), and tuber size (2) One-way ANOVA was conducted at each sample period, and if differences between treatments were detected, means were separated by an $LSD_{0.05}$

Results and Discussion

Study 3. Vertical Distribution of Tubers is Influenced by Mechanical Impedance.

Tuber production in the sand sediment was influenced by the depth of barrier placement up to 27 cm (Table 2 7) With the exception of the deepest barriers placed at 24 and 27 cm, the total number of tubers in the 3 cm interval preceding the barriers were significantly greater than all other 3 cm intervals combined. Total tuber production was reduced in cores containing the barrier at the 3 cm depth compared to all other depths in the sand sediment (Table 2 7) In contrast to the sandy sediments, only 2 viable tubers were found in the 54 cores in which peat sediments were used (Table 2 7) This result was unexpected as no differences in vegetative biomass were noted between the sand and peat cores (5.3 ± 1 4 g dry wt vs 6 1 ± 2 4 g dry wt. respectively) Although rhizome formation was evident in the peat cores throughout the study, no tubers formed. Extensive root systems were found throughout both the organic and sand cores to a depth of 30 cm and visual observations suggested that the Styrofoam barriers did not inhibit root penetration, but did inhibit most rhizomes from penetrating past the barrier

Redox potential varied with time and often between cores Sandy sediments ranged between -113 and +110 mV, while the organic sediments were more reduced and ranged between -194 and +340 mV Although a slight trend toward increasingly reduced sediments over time and depth emerged, due the variation in readings between individual

Table 2.7 Depth of tuber formation in relation to placement of a semi- permeable barrier at 3 cm intervals. Hydrilla was planted in 10 cm diameter PVC cores, and allowed to form tubers.

Tuber Production at 3 cm Interval in Sand Sediment

Barrier Depth (cm)	3	6	9	12	15	18	21	24	27
3	9.6*	0.1	0.2	-	-			-	-
6	2.2	15.5*	1.3	-	0.1	-			-
9	1.1	2.0	14.1*	0.1	-			-	-
12	0.3	0.6	3.3	14.4*	0.2	-			-
15	1.2	0.1	1.1	2.6	15.3*	0.2		-	-
18	0.2	0.3	1.1	1.4	4.2	13.5*			
21	0	0.6	0	0.5	1.1	3.6	12.9*		-
24	1.2	1.0	0.3	1.6	2.3	4.0	5.1	8.3	-
27	0.2	1.4	0.1	0.5	0	1.3	2.0	5.9	6.9

Tuber Production at 3 cm Interval in Organic Peat Sediment

3	0.1				-	-	-	-	-
6	0	0	-	-	-	-	-	-	-
9	0	0	0	-	-	-	-	-	
12	0	0	0	0	-	-	-		
15	0	0	0	0	0	-	-	-	-
18	0	0	0	0	0	0	-	-	
21	0	0	0	0	0	0	0	-	
24	0.1	0	0	0	0	0	0	0	
27	0	0	0	0	0	0	0	0	0

* Tuber values in 3 cm intervals immediately above the barrier depth were different ($LSD_{0.05}$) from all other treatments (n=6)

cores, no attempt was made to correlate tuber distribution with sediment redox potential. Sediment pH differed between the peat (5.0 ± 0.9) and sand cores (7.3 ± 0.85) throughout the study. No differences were detected in sediment pH with increasing depth (data not shown). This study confirms that depth of tuber formation was determined by mechanical impedance in the homogenous fine sandy sediments. In many cases, tubers

were observed to be directly imbedded in the Styrofoam disks Some rhizome penetration past the Styrofoam barriers was noted, presumably due to the fact that the inserts were either not completely sealed at the edges or completely impermeable to rhizome penetration.

Results obtained with the organic peat sediments were perplexing, as plant biomass was not impacted, but tuber production was inhibited It was suspected that either sediment pH may have been too low for tuber production or the release of organic leachates from the peat may have been directly toxic to the rhizomes. No discernable impacts were noted on hydrilla roots in the organic peat sediment

Study 4

Depth of tuber production in the sand and potting soil sediments was once again determined by the depth of barrier placement up to 24 cm deep (Table 2 8) Although tuber values in the potting soil at the 24 and 27 cm barriers were greater than at other 3 cm intervals , these values were not significantly different from all other 3 cm intervals, suggesting that by 24 to 27 cm, sediment depth was becoming a limiting factor to rhizome growth Increased tuber production in the sand sediments compared to the first study was attributed to improved vegetative growth and the fact that cores were grown outdoors for 9 months, rather than indoors for 4 months During harvest, it was noted that large aggregates (5-20 mm diameter) had formed in the potting soil following flooding (small samples suggested that up to 30% of the soil mass was in the aggregate form), and thus may have impacted rhizome penetration. Data from studies 3 and 4 consistently demonstrate that mechanical impedance determines the depth of tuber formation to depths of 24 cm, where rhizome growth is likely limited by other factors

Table 2 8 Depth of tuber formation in relation to placement of a semi-permeable Styrofoam barrier at 3 cm intervals in study 4 Hydrilla was planted in 10 cm diameter PVC cores, and allowed to form tubers

	Tuber Production at 3 cm Interval in Sand Sediment								
Barrier Depth (cm)	3	6	9	12	15	18	21	24	27
3	9 6*		0 5		-	-	-	-	
6	4 2	25.4*	3 3		-		-	-	-
9	2 0	3 6	20 9*	1		-	-	-	-
12	2.3	1 2	5 3	24.4*	0 4		-	-	-
15	3.2	2 0	5 1	1 6	21 0*	2 2	0 2	-	-
18	1.2	3 3	0 1	2.4	6 4	18 5*	-	-	-
21	0	2 7	2 3	1 5	3 0	5 1	17 7*	-	-
24	2.2	1 0	1 3	0 6	2.3	5 0	8 1	13 3*	-
27	0.2	2 4	0 1	3 1	1 6	5 3	5 0	7 9	10 4
	Potting Soil								
3	11.9*	0 3	-	-	-	-	-	-	-
6	2 0	27 1*	1 1	0.2			-	-	
9	2.7	6 0	20 1*	-	0 1	-	-	-	-
12	1 6	4 2	6 6	19 5*	-	-	0 1	-	-
15	1 9	3.2	4.4	5 1	15 1*	0.3	-	-	-
18	1 1	2 3	5 1	3 0	4 1	14.4*			
21	1 6	0 6	4 9	3 1	2.5	5.5	12 9*	-	-
24	0 8	3 3	1 9	2 2	4 1	2 7	6.8	9 8	-
27	0.3	2.2	1 4	2 6	1 6	2 1	5.5	8 9	5 1
	Organic Peat Soil								
3	0 1	-	-	-	-	-	-	-	-
6	0.4	0	-	-	-	-	-	-	
9	0 4	0	0		-				
12	0 3	0	0	0	-	-	-	-	
15	0	0	0	0	0	-	-	-	-
18	0 1	0	0	0	0	0	-	-	-
21	0	0	0	0	0	0	0	-	-
24	0 6	0	0	0	0	0	0	0	
27	0	0	0	0	0	0	0	0	0

*Tuber values in 3 cm intervals immediately above the barrier depth were different ($LSD_{0.05}$) from all other treatments (n=6)

As was noted in study 3, only a few viable tubers were formed in cores containing organic peat sediments (Table 2 8) Nevertheless, comparisons between the three sediment types indicated that vegetative biomass was actually greater in the organic peat (7 3 ± 1.2 g dry wt/core) when compared to the sand (4 6 ± 1 6 g dry wt /core) and potting soil (4 8 ± 2 1 g dry wt /core) sediments Evidence of rhizome formation was found in all organic cores, however, all of these rhizomes were found to be rotten at approximately 4 cm of depth. In contrast to rhizomes, roots were found to be thriving in the organic sediments all the way to the base of the PVC core (30 cm in depth)

Sediment redox readings were more consistent than noted in study 1, with a slight trend towards more reduced sediments as depth increased. Differences were noted between the sediment types as reducing conditions increased from sand (-47 to -135 mV) to potting soil (-265 to -330 mV), to organic peat (-301 to -355 mV) Results suggest that sediment redox was not impacting depth of tuber formation, nor was redox potential too low for tuber production in the organic peat substrate As noted in study 1, sediment pH in the organic peat (4 9 ± 1 1) was significantly reduced compared to the other sediments (potting soil = 6 9 ± 0 8, sand = 7 4 ± 0 9)

Results of sprouting studies from the herbicide treated cores indicated that sprouting rates remained low in both sand and potting soil sediments throughout the sample period (Table 2 9) Although differences in sprouting were noted between sediment depths, no clear trends existed to suggest that vertical position of the tuber impacted potential for sprouting Moreover, differences were noted between sprouting rates in the sand vs. potting soil sediments at given depths, however, no trends emerged to suggest increased sprouting in one medium compared to another (Table 2 9).

Table 2 9 Percent sprouting nf tubers at defined depth intervals in PVC cores following removal of vegetative growth above the sediment Styrofoam barriers were placed at 3 cm intervals to concentrate tubers at each depth interval

	Tuber Sprouting (%)			
	60 Days		120 Days	
Barrier Depth, cm	Sand	Potting Soil	Sand	Potting Soil
3	19*	12	21	22
6	18	16	27	23
9	13	17	11	17
t2	7	10	21	24
t5	11	12	17	23
18	8	18	16	33
21	17	8	26	14
24	8	16	t9	7
$LSD_{0.05}$	5 6	4 7	5 1	4 8

*Each value represents the average nf 3 replicate cores

These results confirmed observations from study that depth nf tuber formation is determined by mechanical impedance The more random distribution noted in the potting soil as compared to the sand was attributed to increased heterogeneity (particle size) noted in the potting soil. It is suspected that large aggregates formed in the potting soil inhibited rhizome penetration The lack of tuber production in the organic peat confirmed results of the first study, and this lack of production was even more perplexing as the organic peat cores had nearly double the biomass noted in the sand and potting soil cores Tuber sprouting was not impacted by depth in the sediment profile in either the sand or potting soil, and was not related to redox potential Subsequent viability tests, indicated that >95% nf these tubers sprouted within 1 week following their removal from the sediment, again confirming an environmentally-imposed quiescence.

Study 5. Filtering Sediments Impacted Tuber Distribution

Temperature measurements taken at several times and dates, showed that the heating element elevated water temperatures 7 to 9 C above ambient temperatures. These increased water temperatures stimulated vigorous hydrilla growth, resulting in a five-fold increase in tuber production compared to a set of cores placed in a non-heated vault.

Tuber production in the organic peat sediment was very low, with only a few viable tubers found in all of the cores harvested (data not shown). Tuber production and vegetative growth (6 9 ± 1 9 g dry wt /core) was prolific in cores filled with Orange Lake sediments, however, at depths below 9 cm, tuber distribution differed between filtered and unfiltered sediments (Table 2 10) The majority of tubers formed at the barriers through 24 cm Results suggest that large particulates in the unfiltered sediment impacted rhizome penetration and therefore depth of tuber distribution Sediments remained highly reduced throughout the study (-337 mv ± 57) and pH was measured at 6 9 ± 1 1

Low rates of sprouting were observed in both the filtered and unfiltered cores at 60 and 180 days (Table 2.11) No differences in sprouting were detected within depth increments or between sediments, nor apparently as function of redox potential or pH The low rates of sprouting and distribution of tubers throughout the cores made it difficult to ascertain the ability of tubers at different depth intervals to emerge to the sediment surface. In this study, removing the vegetative canopy with a herbicide treatment did not appear to stimulate tuber sprouting, however, there were no untreated controls to compare to these sprouting rates.

Table 2.10 Depth of tuber formation in filtered and unfiltered Orange Lake sediments in relation to placement of permeable barriers at increasing 3 cm depths.

	Tuber Production at 3 cm Interval								
Barrier Depth (cm)	3	6	9	12	15	18	21	24	27)
Filtered Sediment									
3	16.6*	0.1	0.2	-			-	-	-
6	3.2	26.5*	3.3	0.1					
9	0.1	1.6	34.1*	0.8	0.2		-		-
12	0.0	1.5	2.3	25.5*	-	0.5			
15	1.0	1.1	0.8	4.3	25.1*	0.4	-	-	-
18	0.4	1.1	1.5	0.4	2.2	27.0*	-	0.2	
21	1.1	0.8	1.4	3.2	0.8	3.8	20.2*	-	-
24	1.2	2.0	0.6	2.1	1.3	3.0	6.1	14.3*	-
27	0.2	1.7	2.1	1.1	2.2	2.3	4.1	4.8	8.0
Unfiltered Sediment									
3	19.1*	3.8	0.6			-	-	-	
6	0.6	24.2*	-	0.2	-	-	-	-	
9	1.4	4.2	25.2*	0.9	-	0.1	-		-
12	1.1	4.6	2.4	21.6*	0.5	-	-	-	-
15	1.8	6.1	3.1	4.2	17.1*	0.4	0.2		
18	2.0	1.3	4.8	2.1	3.3	17.7*	-	-	-
21	1.1	3.1	0.9	3.9	5.1	4.4	15.4*	0.2	
24	2.1	0.7	2.5	3.1	3.3	4.1	6.0	8.9	-
27	1.8	3.3	2.9	2.1	3.8	4.1	6.1	3.4	7.1

*Tuber values at the barrier depth were different ($LSD_{0.05}$) from all other treatments (n=6)

Table 2 11 Percent sprouting of tubers at defined depth intervals in PVC cores collected from Orange Lake, FL Styrofoam barriers were placed at 3 cm intervals to concentrate tubers at each depth interval

	60 Days		120 Days	
Barrier Depth, (cm)	Filtered	Unfiltered	Filtered	Unfiltered
3	7*	6	11	12
6	5	11	11	17
9	8	7	13	15
12	7	10	9	14
15	5	11	13	15
18	10	8	10	11
21	8	8	14	12
24	6	6	15	11
$LSD_{0.05}$	NS		3.4	

*Each value represents the average of 6 replicate cores

Study 6. Sediment Manipulation Influenced Sprouting Rates.

The majority of tubers in undisturbed sediment and sediment in which the top 6 cm were removed remained quiescent by 90 d (Table 2 12) The low sprouting rates observed in the undisturbed cores were similar to those observed in studies 3 and 4 In contrast, partially exposing the tubers (1-3 mm of sediment) resulted in a significant increase in sprouting (Table 2 12) Although differences in sprouting rates were noted between the studies, sprouting trends remained similar between treatments It should be noted that it was difficult to duplicate removal of sediment to partially expose the tubers, and some variability likely existed within the replicates While redox readings showed that sediments remained reduced in the undisturbed cores and cores from which the top

Table 2.12 Percent hydrilla tuber sprouting in PVC cores following physical manipulation of sediments to alter the vertical position of the tuber

	% sprouting at 90 days
Study 1 (August 1997)	
Undisturbed	16 b*
1-3 cm sediment	19 b
1-3 mm sediment	59 a
Study 2 (August 1998)	
Undisturbed	4 c
1-3 cm sediment	15 b
1-3 mm sediment	41 a

*Different letters following each value indicate significant differences between treatments according to an $LSD_{0.05}$ (n=10)

6 cm of sediment were removed, readings were highly variable (+ 390 to -104 mV) in the cores in which partial exposure was attempted (especially in study 2). Obtaining consistent readings in these cores was quite difficult, and it is likely that a range of exposure to light and oxidized conditions were experienced by the tubers near the sediment-water interface.

Study 7. Increasing pH of Organic Peat Sediment Increased Tuber Production.

Amending sediments with lime increased sediment pH above 6.5 and all lime treatments resulted in a significant increase in tuber production compared to unamended sediments (Table 2.13). In addition, no differences in biomass or tuber production were noted between new organic peat sediments and those re-used from a previous study. Sediment redox was consistent (-275 to -351 mV) between treatments. Results suggest

Table 2 13 Effect of lime addition on organic peat sediment pH, hydrilla biomass, and tuber production. Pots were established in November and harvested in May

Treatment	pH	Biomass (g dry wt/pot)	Tubers/pot
New Peat			
No Lime	5 6 b*	3 8	2 c
1% Lime	6 9 a	4.2	18b
2.5% Lime	7.2 a	3 7	23a
5% Lime	7.2 a	4.0	25 a
Used Peat			
No Lime	5 8 b	4 3	5 e
1% Lime	7 1 a	4 0	23 a
2 5% Lime	7.2 a	3.5	20ab
5 0% Lime	7 4 a	3 8	26a

*Values followed by different letters indicate differences existed between treatments according to an $LSD_{.05}$ (n=6) Data for the two studies was combined for analysis.

that sediment pH did not impact plant biomass, but the low pH in the unlimed organic peat sediments did result in decay of rhizomes and prevented formation of viable tubers Increasing pH overcame this effect. The mechanism of the toxicity was not determined, however, Ponnamperuma (1972) has suggested that a pH change of 0 5 units can mean the difference between metal toxicity or deficiency for rice in a flooded soil and increasing pH of acid soils has long been known to eliminate the toxicity of aluminum to rice Organic soils that have a low cation exchange capacity (especially if they are fertilized) are likely to result in the presence of too much salt for the healthy growth of rice (Ponnamperuma 1972) In peat soils, organic acids have been shown to be toxic to

rice culture at pH values below 6 0 (Ponnamperuma 1972) While sediment pH has generally not been reported to impact tuber production, these results suggest that in some areas in which hydrilla growth is supported, low pH sediments could reduce tuber production The extreme disjunct between biomass accumulation and reduced tuber production has not been noted in previous studies Spencer and Ksander (1995) demonstrated that addition of acetic acid to sediments results in significant tuber mortality Although the authors demonstrate significant electrolyte and amino acid leakage from the treated tubers, these studies did not characterize sediment pH following addition of acetic acid. The mechanism of toxicity due to the low pH remains unknown, but could be due to increased metal toxicity (Ponnamperuma 1972), or direct toxic effects from release of an organic acid (Spencer and Ksander 1995)

Study 8. Depth of Planting Influenced Emergence to the Sediment Surface

Data collected from plexiglass chambers in which pre-sprouted tubers were planted along a 45 degree gradient showed that percent tuber emergence was impacted by all main treatment effects (sediment type, sediment depth and propagule size), however, a significant 3-way interaction was also indicated Depth of planting influenced both the timing of emergence and ability of the tuber to emerge to the sediment surface. To facilitate interpretation, tuber emergence data were consolidated into 3 cm intervals. Emergence of tubers through the builders sand was extremely limited at depths greater than 8 cm Observations suggested that emerging apical tips were being damaged by the coarse sand at approximately 2-4 weeks after treatment. ANOVA results indicated that the two studies were significantly different, however, if the sand data is not included in the analysis, ANOVA suggested that studies 1 and 2 were not significantly different

(potting soil and Orange Lake sediment) Therefore, potting soil and organic soil results were combined for statistical analysis, whereas data collected for the Austin Cary sediment is from the second study only One-way ANOVA indicated differences in the timing of emergence noted between the sediments with tubers emerging readily in the soft Orange Lake organic sediment compared to potting soil, and Austin Cary (sand) sediments (Table 2 14) In most cases, tuber emergence was slowest in the dense Austin Cary sediments As tuber depth increased, the time required to emerge to the sediment surface also increased, with the larger size class of tubers (350-450 mg) much more likely than the smaller tubers (100-200 mg) to emerge from depths greater than 16 cm in all sediment types (Table 2 14)

The reduced density and homogenous nature of the Orange lake sediments (0 82 ± 0 11 g/cc) compared to the aggregates formed in the potting soil (0 95 ± 0 12 g/cc) and the sandy Austin Cary sediments (1 19 ± 0 08 g/cc) likely allowed more rapid elongation of sprouted tubers due to reduced physical resistance In the case of the coarse builders sand (1 53 ± 0 12 g/cc) growing tips were not able to penetrate this dense medium While sediment density likely influenced timing of emergence, the influence of sediment redox potential on stem elongation can not be disregarded Redox probes placed in the plexiglass chambers suggested that the Orange lake sediments were more reduced (-389 ± 31 mV) than either the potting soil (-271 ± 24 mv) or Austin Cary sediment (-245 ± 38 mV) Research by Summers and Jackson (1995) have suggested that *Potamogeton pectinatus* tubers are stimulated to elongate under completely anaerobic (i e reduced) conditions While this phenomenon has not been demonstrated for sprouting hydrilla tubers, it suggests that shoot elongation may be influenced by sediment redox potential

Table 2 14 Influence of depth of planting on hydrilla tubers of 2 size classes (100-200 mg and 350-450 mg) on their ability to emerge to the surface in three sediment types Tubers were pre-sprouted prior to planting in plexiglass chambers.

	Tubers Emerging to Sediment Surface (%)								
	Weeks After Sprouting								
	1	2	4	6	8	10	12	14	16
100-200 mg size Class									
1-4 cm Ps*	33	92	100	100	100	100	100	100	100
1-4 cm Org	53	94	100	100	100	100	100	100	100
1-4 cm Ac Snd*	25	57	57	88	94	94	94	94	94
5-8 cm Ps	0	34	44	73	89	89	89	89	89
5-8 cm Org	0	47	75	83	94	94	94	94	94
5-8 cm Ac Snd	0	17	38	47	68	88	92	92	92
9-12 Ps	0	0	29	46	63	72	92	92	92
9-12 Org	0	37	68	83	93	92	92	92	92
9-12 Ac Snd	0	0	15	29	45	0	0	0	0
13-16Ps	0	8	8	17	38	75	75	75	75
13-16 Org	0	8	20	35	55	83	83	83	83
13-16 Ac Snd	0	0	0	10	23	0	0	0	0
17-20 Ps	0	0	0	17	25	33	58	58	67
17-20 Org	0	0	11	31	35	33	58	70	77
17-20 Ac Snd	0	0	0	7	17	0	0	0	0
20-23 Ps	0	0	0	8	8	8	17	25	31
20-23 Org	0	0	0	8	17	17	25	25	25
20-23 Ac Snd	0	0	0	0	4	0	0	0	0
> 23 Ps	0	0	0	0	0	0	0	0	0
> 23 Org	0	0	0	0	0	0	8	8	8
> 23 Ac Snd	0	0	0	0	0	0	0	0	0
350-450 mg Size Class									
1-4 cm Ps*	63	98	98	98	98	98	98	98	98
1-4 cm Org	74	94	94	98	98	98	98	98	98
1-4 cm Ac Snd	25	57	73	82	88	94	94	94	94
5-8 cm Ps	20	50	69	77	91	91	91	91	91
5-8 cm Org	33	65	92	92	96	96	96	96	96
5-8 cm Ac Snd	26	35	58	70	87	87	87	87	87

Table 2 14 Continued

	Tubers Emerging to Sediment Surface (%)								
	Weeks After Sprouting								
	1	2	4	6	8	10	12	14	16
350-450 mg ane Chas									
9 12 Ps	0	8	36	48	63	72	92	92	92
9-12 Org	0	29	68	83	92	92	92	92	92
9 12 Ac Snd	0	7	25	55	60	74	82	90	90
13 16 Ps	0	7	11	36	49	73	85	89	89
13-16 Org	0	24	31	55	64	75	86	86	86
13-16 Ac Snd	0	7	16	29	39	59	77	82	82
17 20 Ps	0	0	11	17	45	53	73	84	89
17-20 Org	0	19	21	39	51	70	84	86	86
17 20 Ac Snd	0	0	8	18	23	39	59	72	78
21-24 Ps	0	0	0	23	28	39	51	65	72
21-24 Org	0	0	9	33	47	59	68	77	77
21 24 Ac Snd	0	0	0	18	18	31	60	73	60
25-28 Ps	0	0	0	18	18	27	40	40	40
25-28 Org	0	0	7	13	21	38	49	57	61
25-28 Ac Snd	0	0	0	11	14	22	29	35	46
29-30 Ps	0	0	0	0	11	19	26	33	33
29-30 Org	0	0	8	13	18	18	29	39	46
29-30 AcSnd	0	0	0	0	12	17	20	29	35
LSD 0 05[1]	10	9	7	12	7	9	11	8	13

[*] Ps= potting soil Org= Orange Lake organic sediment, and Ac Snd = Austin Cary Sand

[1]Data from 2 studies were pooled with the exception of AcSnd which was only included in the second study

[2]$LSD_{.05}$ - Comparison of percent emergence within each sample period for all treatments

Based on these results, increasing sediment depth, the sediment type (density), and tuber size all impact the timing and ability of a tuber to emerge from the sediment following sprouting. Interestingly, a large number of sprouted tubers in all sediment types did not emerge during the 4 month study. This is in contrast to reports by Mitra (1956) that propagules emerge within 1 to 2 weeks of sprouting. Although these tubers were extremely etiolated, they remained viable propagules (once exposed to light, they developed normally). Furthermore, it was noted that sprouted tubers sometimes grew in a spiral nature, and this behavior likely reduced their chances to grow to the sediment surface. Although Bowes et al. (1977) and Spencer and Ksander (1996) have suggested that tuber storage reserves may become depleted within a 4 month period, their studies were conducted in aerobic environments, and it is likely that hydrilla tubers in this study remained in an anoxic environment. Anaerobic metabolism may allow sprouted tubers to remain viable (capable of emerging) for a much longer period than has been postulated.

The ability to visualize the behavior of sprouting tubers in the plexiglass chambers suggested that this experimental apparatus may have further utility for future propagule studies. Visualizing tubers difficult in organic sediments compared to sandy sediments, but further research with these or similar chambers should be considered.

Summary and Conclusions

Collection of tubers from the drawdown sites at Rodman Reservoir indicated that tuber sprouting and decomposition was greater in sandy sediments (oxidized during the drawdown) following submersion, whereas, organic sediments remained reduced and a large percentage of tubers were still quiescent up to 1 year later. In several of the sand

dominated sample sites, tuber distribution in the sediment reached a distinct maximum followed by a rapid decline. Subsequent research suggested that the depth of tuber formation is largely determine by mechanical impedance. Nonetheless hypotheses suggesting that the general lack of tubers found in the upper 6 cm of sediment were due to increased sprouting rates at these shallow depths were not validated. Manipulating sediments to change the vertical position of tubers did not influence sprouting (unless tubers were physically exposed). Although a changing environmental gradient is expected with increased sediment depths, there were no indications that tuber sprouting was influenced by vertical position in the sediment. Studies conducted with a commercial peat soil resulted in excellent vegetative growth of hydrilla and formation of numerous rhizomes, however, low sediment pH (<5.5) resulted in decomposition of rhizomes prior to tuber formation. Amending these organic sediments with lime to adjust the pH (>6.5) resulted in prolific production of tubers. The mechanism of the pH toxicity was not described. Studies conducted in plexiglass chambers indicated that tuber depth in the sediment, tuber size, and sediment type all impacted the timing of emergence. Interestingly, many tubers that did not emerge to the sediment surface during the course of the study remained viable in the sediment 4 months after sprouting.

CHAPTER 3
MESOCOSM EVALUATIONS IMPACT OF MANAGEMENT TECHNIQUES ON THE SPROUTING OF DIOECIOUS HYDRILLA TUBERS

Introduction

Sculthorpe (1967) suggests that hydrilla evolved in a monsoonal climate and that tubers act as survival structures during the dry period and upon flooding are stimulated to sprout This observation would explain why artificial drawdowns of lakes and reservoirs in the United States have resulted in a significant stimulation of sprouting of hydrilla tubers, particularly in sandy hydrosoils (Miller 1975, Haller and Shireman 1983) Nonetheless the use of drawdowns to manage hydrilla is often not practical in many flood control and multi-purpose use aquatic systems Moreover, due to the significant stimulation of tuber sprouting following re-flooding of drawdown sites, secondary measures to control sprouting tubers are necessary to prevent rapid reinfestation by hydrilla. To date, drawdowns are the only control measure that has been definitively shown to significantly increase hydrilla tuber sprouting, but the length of time required for a drawdown to stimulate tuber sprouting remains unknown

Several authors have suggested that sprouting of tubers is greatly stimulated following various chemical and mechanical methods to control hydrilla (Mitra 1955, Van and Haller 1979, and Joyce et al. 1992) Increased light penetration through the water column, changes in sediment temperature, and changes of the gaseous constituents of the sediments have all been suggested as possible triggers to stimulate sprouting. While data

which determine factors involved in stimulating tuber sprouting are scarce, it should be noted that there is also scant quantitative evidence to support the contention that tuber sprouting is greatly increased following hydrilla management. Data are lacking both in terms of timing and magnitude of tuber sprouting following application of management techniques, as well as the rate of sprouting in comparison to untreated systems of a similar nature.

While management is thought to stimulate sprouting, laboratory studies have suggested that tubers of the monoecious biotype of hydrilla remain quiescent until exposed to a cold period during the winter months (Carter et al. 1987, McFarland and Barko 1995). In contrast, the vast majority of research has been conducted with dioecious hydrilla tubers in Florida, where it is unlikely that exposure to cold conditions occurs for a considerable length of time. It remains unknown if the sprouting of dioecious hydrilla tubers will increase if the tubers are exposed to colder winter temperatures.

A series of mesocosm studies was conducted in order to address questions concerning the impact of management techniques on the sprouting of hydrilla tubers. Management is associated with the removal of a vegetative canopy, and it is likely that light penetration and sediment temperature notably increase in aquatic systems (<4 m in depth) where hydrilla has been treated. In addition, it has been hypothesized that changes in sediment redox potential following removal of the vegetative canopy may stimulate tuber sprouting. The objective of these studies was to better quantify tuber sprouting following various management techniques, as well as provide information on specific factors influencing *in situ* tuber sprouting. In addition, short-term drawdowns (< 14 d)

were simulated in the mesocosms to determine the length of time required for a drawdown to stimulate tuber sprouting

Materials and Methods

Mesocosm Facility and Experimental System

A series of 38, 900 L concrete vaults (219 cm long x 76 cm wide x 64 cm deep) located near Bivens Arm Lake in Gainesville, FL, were used to conduct mesocosm studies over a period of three years Unless otherwise noted, plastic flats (11 L capacity, 35 cm long x 31 cm wide x 14 cm deep dishpans) were filled with commercial potting soil (Lilliston) amended with 3 g of Osmocote (20-5-8) per kg of soil, and covered with a 2 cm layer of builders sand to minimize sediment suspension during handling. Twelve shoot apices of hydrilla were planted in each flat and flats in flats were then placed in concrete vaults on 1 August or early September During the course of all studies, hydrilla established rapidly and filled the capacity of the vaults within one month of planting Well water was allowed to flow through the tanks at an approximate rate of 1 replacement every 48 to 60 hours Tubers formed from late September thru May

A Campbell CR10X Datalogger (Campbell Data Systems, Logan Utah) was programmed to record temperature at 4 hour intervals. Thermocouple probes were placed 10 cm below the water surface and at a depth of 0, 4, and 10 cm in the sediment for each treatment. In addition, redox probes (described in Chapter 2) were deployed at sediment depths of 0, 2, 8, and 12 cm in 3 flats of each treatment vault and reduction potential recorded with a hand-held pH/mV meter on a daily basis for 21 days and weekly thereafter for the remainder of the study Light measurements at the water and sediment

surface were recorded with a LiCor quantum scalar irradiance meter on a weekly basis during the course of the study

Study 1 – The Impact of Canopy Removal on Tuber Sprouting

Hydrilla was established in 24 vaults in September of 1995. On July 1, 1996, one flat was removed from each vault to provide estimates of pretreatment biomass, as well as the total number of tubers that had formed and sprouted during the previous fall, winter, and spring.

Treatments that were applied in July of 1996 included the following: 1) untreated controls, 2)endothall applied at 5.0 mg/L, 3)chelated copper at 3.0 mg/L, 4)endothall at 5.0 mg/L followed by cover of the surface water with a detached artificial plant canopy, and 5) mechanical clipping of plants to < 15 cm above the sediment surface every other week. Each treatment was replicated three times and vaults were treated in a completely randomized design.

In the case of the endothall and copper treatments alone, the goal was to rapidly remove all of the surface vegetation and determine if any differences in tuber response might exist following the use of different contact herbicides. These vaults were retreated 3 weeks following initial application to ensure that all surface vegetation was removed.

The endothall treatment followed by the use of the detached canopy was conducted to remove the existing surface vegetation and yet simulate and maintain sediment temperatures and light levels that are experienced under the vegetative mat of hydrilla. This treatment was conducted to test the hypothesis that light penetration and sediment temperature play a role in stimulating tuber sprouting. To simulate a vegetative canopy of hydrilla, a fine mesh fabric (2 mm) was secured in the vaults at a depth of 15

cm below the water surface and then covered with detached hydrilla stems. Hydrilla stems fared quite well on the mesh barriers and were only replaced infrequently as the detached plants rooted extensively through the mesh and generally produced healthy new growth. In the case of herbicide treatments that were followed by placement of an detached canopy, water was used to flush the tanks several times at 3 thru 5 days posttreatment to remove residues prior to placing detached hydrilla stems on the mesh.

The objective of the mechanical clipping treatments was to remove the canopy effect and yet maintain a viable hydrilla shoot and root system. By removing the canopy, sediment temperatures and light penetration would likely increase. As with the use of the artificial canopy, this treatment was conducted to test the hypothesis that increased light penetration and sediment temperature influenced sprouting of quiescent tubers. The canopy was clipped back every 2 weeks to maintain plants near the sediment surface.

Following the July 2 treatment, two flats were harvested from each vault at 2, 4, 8, 12, and 20 weeks. Total tuber number was quantified as well as the percent sprouting and rotting. In addition, shoot and root biomass was also collected. Non sprouted tubers were placed in petri dishes to determine percent viability following each harvest.

Data were statistically analyzed by ANOVA and if differences between treatments were detected, data were further subjected to regression analysis. A t-test$_{0.05}$ was used to determine differences between treatments harvested on the same date.

Study 2 - Effect of Canopy Removal, Sediment type, and Experimental Container on Tuber Sprouting

Study 2 was initiated in August 1996, and several treatments from the Study 1 above were repeated. In addition, a complimentary set of flats consisting of 85% builders

sand and 15% potting soil was used for treatments which included untreated controls, endothall at 5 0 mg/L, endothall at 5 0 mg/L + artificial canopy and mechanically clipped plants Thus allowed comparison af tuber response to various forms of management in the potting soil versus a sand sediment As noted in the prior Chapter, sprouting differences were noted in sand and organic sediments from Rodman Reservoir

In addition, to the plastic flats, 30 cm tall pvc cores (10 cm diameter) described in Chapter 2 were filled with potting soil and 4 apices of hydrilla were planted in August 1996 Treatments included untreated controls, endothall at 5 0 mg/L, endothall at 5 0 mg/L + artificial canopy, and mechanically clipped plants The PVC cores were used to prevent the artificially close association of hydrilla roots and tubers that was observed in the plastic flats used in the first study A Styrofoam insert was placed at the bottom of each pvc core. Tuber sprouting in PVC cores was compared to sprouting in plastic flats

Data analyses included use of ANOVA to compare tuber response to management in studies 1 and 2 (using the potting soil media in plastic pans), tuber response to the different soil media (potting soil vs sand in study 2), and tuber response to different container types (plastic flats vs PVC cores). If differences between treatments were detected data was further subjected to regression analysis

On May 26 1997 one flat was removed from each treatment (36 containers) to provide pretreatment biomass and tuber data Treatment of hydrilla in plastic flats and PVC cores was conducted on May 27, 1997, and temperature, redox, and light data were collected as described for the first study Posttreatment harvests included both shoot and root biomass, and collection of tuber data as was described for study 1

Study 3 Impact of Tuber Age on Potential For Sprouting

Study 3 was initiated in August 1997 and included flats of untreated plants that had been established during 1995, 1996, and those just planted in 1997. Flats from 1995 provided tubers that were of 3 different age classes, those from 1996 contained two different age classes, and those from 1997 contained newly formed tubers. Treatments included untreated controls, 5.0 mg/L endothall, and plants that were mechanically clipped. On June 4, 1998, six flats were harvested from each of the 3 age classes, and rootcrown density, shoot biomass, and total tuber number per flat were quantified.

Vaults were treated on June 5, 1998 and tuber data and shoot biomass data were collected at 8 and 16 weeks. Total tuber numbers and percent sprouting were compared for the 3 age classes of tubers. Data were subjected to ANOVA and percent sprouting results compared within each age class and between age classes.

Study 4 - Influence of Duration of a Drawdown on Tuber Sprouting

While the sprouting response of tubers following long-term drawdowns is well documented, it remains unclear if short-term drawdowns could have a similar effect. In order to determine if short-term drawdowns could impact tuber sprouting, 95, 10 cm diameter PVC cores (described in Chapter 2) were filled with potting soil (30), 85% builders sand + potting soil (30), or sediment collected from ponds at the Austin Cary Forest (30). The bottom of each core contained a styrofoam insert to allow water trapped in the core to drain. Hydrilla was established in August 1996, and tubers formed thru May 1997. Twelve cores were removed from the vaults on May 26 and exposed to drying conditions for 0 (5minutes), 1, 2, 7, and 14 days. Redox at 4 and 12 cm was recorded at each sampling period and at 24 and 48 hrs following re-submerging the cores

Following the drying period, cores were reflooded and six cores from each treatment were harvested at 30 and 60 days posttreatment. Total tuber number and percent quiescent, rotted, and sprouted tubers were quantified and compared among treatments. In addition, shoot biomass was recorded for each treatment at the 30 and 60 day harvests.

This study was repeated in August 1997 thru August 1998. Cores were removed from the vaults on June 15, 1998. Treatment effects of the two studies were compared by Analysis of Variance and no significant differences were found. Therefore data from the two studies were pooled for ANOVA and if treatment differences were detected, means were separated using an $LSD_{0.05}$.

To further test the impact of drawdowns, a set of 50 PVC cores filled with potting soil was planted with hydrilla in August of 1997 and allowed to form tubers through May 1998 as described above. Prior to planting the hydrilla, Styrofoam barriers (described in Chapter 2) were placed at a 15 cm depth in 25 of the cores and at the bottom (30 cm) of the remaining cores. On June 15, 1998, the water level in the vaults containing these cores was dropped to a depth of 15 cm. Water levels were maintained by placing a stand pipe in the vault and dripping water into the vault to compensate for evaporation rates. The upper 15 cm of the PVC cores were thus exposed to drying conditions that resulted in rapid dessication of the shoot biomass. Redox probes were placed in the cores at depths of 10 and 18 cm. Ten cores representing each treatment were harvested at 30 and 70 days, and tuber number and percent sprouting were quantified. During harvests, data were separated into those tubers occupying the upper and lower 15 cm of each core.

Sprouting data comparing tubers in the upper 15 cm to the lower 15 cm of the cores was subjected to ANOVA. In addition, sprouting data was also compared to data

collected from the 7 and 14 day drawdown studies described above If differences were detected, means were separated by an $LSD_{0.05}$

Results and Discussion

Studies 1 and 2 -Canopy Removal is not Responsible for Increased Tuber sprouting.

Pretreatment data collected for studies 1 and 2 in July of 1996, and May 1997 indicate that biomass and tuber production were not different between studies, and greater than 95% of all tubers remained quiescent prior to application of treatment (Table 3 1) Data collected for the first study between May and July indicate very little change in the percent tuber sprouting during this period Removing these tubers from the sediment and placing them in petri dishes, stimulated > 96% sprouting within 7 days.

Following herbicide treatments most of the hydrilla stems were brown and waterlogged and laying on the bottom sediments within 2 weeks after treatment (WAT) Due to the high use rates and subsequent static exposures, these treatments resulted in near complete control of vegetative biomass in the water column. Retreatment at 3 and 8 weeks ensured residual control Mechanically clipped plants continued to produce new shoot growth that was clipped back every 2 weeks Untreated plants maintained a dense canopy and consistent biomass throughout the duration of the studies (Table 3 2)

Following removal of the vegetative canopy by herbicide and mechanical clipping treatments, sediment temperature and light intensities measured at the sediment surface increased significantly above untreated controls (Tables 3.3 and 3 4) In contrast, the artificial canopy allowed close simulation of sediment temperature and light profiles recorded in the untreated controls (Tables 3 3 and 3 4) Both canopies greatly reduced

Table 3.1. Pretreatment shoot biomass and tuber production of hydrilla that had been planted in plastic flats during August of the previous year. The number of quiescent and sprouting tubers were also quantified.

	Shoot Biomass* g dry wt. / flat	# of Tubers per flat	% Quiescent	% Sprouting
Study 1				
May 1996	131 ± 25	147 ± 24	96 ± 1.8	1.9 ± 1.3
July 1996	171 ± 39	133 ± 26	94 ± 1.4	3.2 ± 1.2
Study 2				
May 1997	148 ± 26	151 ± 28	96 ± 1.3	2.1 ± 1.2

*Values based on the average of 15 flats at each pretreatment sample time.

Table 3.2. Hydrilla shoot biomass (g dry wt./container) at harvest dates for Studies 1 and 2 following various herbicide and mechanical control techniques

Treatment	Sediment Type	2 wk	4 wk	8wk	12 wk	24 wk
Study 1						
Untreated	PS	139a*	155a	161a	141a	136a
Endothall	PS	6fg	3c	2d	47b	55c
Copper	PS	15d	4c	25c	51b	90b
Mechanical Clip	PS	43c	30b	49b	41b	42c
Endothall + Art. Canopy	PS	45c	3c	3d	2c	5d
Study 2						
Untreated	PS	166a	145a	153a	171a	149a
Endothall	PS	42b	12c	13c	33b	48b
Mechanical Clip	PS	45b	47b	56b	42b	48b
Endothall + Art. Canopy	PS	31b	4c	7c	11c	6c
Untreated	Sand	133a	121a	141a	131a	133a
Endothall	Sand	55b	4c	16c	41b	66b
Mechanical Clip	Sand	31c	33b	45b	34b	38c
Endothall + Art Canopy	Sand	57b	7c	5c	6c	11d

*Letters represent significant differences between treatments at each sample period according to an $LSD_{0.05}$ (n=3)

Table 3 3 Temperature profiles (degrees Celsius) recorded in the sediment (4 cm depth) at 0800, 1200, and 2000 hrs for selected dates following July (Study 1) and May (Study 2) treatments of mesocosm tanks

Sample Date	Untreated	Endothall	Art. Canopy	Mechanical Clip
		Study 1		
2 WAT				
0800	23	25	22	24
1200	21	28*	22	26*
2000	22	29*	21	27*
4 WAT				
0800	24	26	23	26
1200	21	29*	22	30*
2000	23	33*	22	32*
8 WAT				
0800	24	29*	25	29*
1200	22	33*	23	32*
2000	21	33*	22	31*
12 WAT				
0800	19	21	21	22
1200	21	24	21	24
2000	21	27*	22	28*
		Study 2		
2 WAT				
0800	20	23	20	25*
1200	21	26*	22	28*
2000	21	27*	20	27*
4 WAT				
0800	25	26	25	27
1200	23	32*	21	29*
2000	22	31*	21	31*
8 WAT				
0800	24	27*	23	25
1200	20	34*	21	33*
2000	20	32*	21	33*
12 WAT				
0800	24	26	22	27
1200	22	31*	21	32*
2000	21	31*	22	33*

*Treatments were significantly different from untreated controls according to a Dunnett's test$_{0.05}$ (n=3)

Table 3 4 Light readings (µmol / m² / sec) recorded at the sediment surface at approximately 1300 hrs for selected dates (sunny days) following a July (Study 1) and May (Study 2) treatment of mesocosm tanks

Sample Date	Untreated	Endothall	Artificial Canopy	Mechanical Clipping	Air
			Study 1		
Pretreat	19	280*	31	185*	2110
2 WAT	15	309*	29	244*	2144
4 WAT	21	197*	16	221*	2126
8 WAT	26	283*	11*	173*	2046
12 WAT	29	176*	17	216*	1994
			Study 2		
Pretreat	9	320*°	11	294*	2233
2 WAT	11	311*	16	343*	2242
4 WAT	8	247*	15	191*	2147
8 WAT	25	193*	21	281*	2221
12 WAT	27	266*	14*	303*	2026

*Treatments were significantly different from untreated controls according to Dunnett's test$_{0.05}$ (n=6)

°Variation in endothall-treated and mechanically-clipped tanks is indicative of water quality changes due to phytoplankton blooms

Table 3 5 Sediment redox potential in flats containing a potting soil or sand sediment (0, 2, 8, and 12 cm depths) following various hydrilla management techniques

	(Redox, mv)			
Sample Date	**Untreated**	**Endothall**	**Art. Canopy**	**Mechanical Clip**
		Potting soil (study 2)		
2 WAT				
sed/water	-300	-265	-251	-211*
2 cm	-322	-310	-266	-269
8 cm	-281	-277	-298	-298
12 cm	-277	-311	-259	-245
4 WAT				
sed/water	-330	15*	-87*	100*
2 cm	-342	-290	-266*	-319
8 cm	-311	-295	-263	-299
12 cm	-297	-311	-309	-275
8 WAT				
sed/water	-304	109*	-127*	150*
2 cm	-312	-267	-331*	234*
8 cm	-322	-288	-312	-309
12 cm	-309	-331	-342*	325
12 WAT				
sed/water	-284	141*	-77*	144*
2 cm	-282	-299	-301	-298
8 cm	-302	-305	-277	-284
12 cm	-291	-329*	-323	-309
		Sand (study 2)		
2 WAT				
sed/water	-199	-165	-151	11*
2 cm	-202	-204	-196	-149*
8 cm	-211	-177	-208	-228
12 cm	-189	-203	-239*	-205
4 WAT				
sed/water	-230	98*	-4*	167*
2 cm	-211	-222	-209	-179
8 cm	-208	-265*	-254*	-192
12 cm	-194	-278*	-259*	-222
8 WAT				
sed/water	-264	45*	-105*	110*
2 cm	-212	-247	-238	-214
8 cm	-207	-258*	-242*	-221
12 cm	-189	-238*	-236*	-205
12 WAT				
sed/water	-204	-164*	-113*	124*
2 cm	-222	-169*	-235	-188*
8 cm	-221	-270*	-241	-212
12 cm	-201	-267*	-254*	-218

*Treatments were significantly different from untreated controls at each sample depth and date according to a Dunnett's test$_{0.05}$ (n=3)

Redox probes were placed just above the sediment surface for these readings

light penetration and served as a heat trap during the day, allowing sediment temperature to remain up to 12 C cooler than in treatments which resulted in removal of the canopy Temperature of the surface canopy often approached 40 C during the heat of the day, whereas water temperatures measured in vaults without a canopy rarely exceeded 30 C

Sediment redox significantly increased at the sediment water interface following removal of the vegetative canopy by 4 WAT, however, readings at 2, 8, and 12 cm depths in the potting soil suggested that sediments remained reduced regardless of the treatments (Table 3.5) In contrast, the sandy sediments were consistently more reduced than untreated controls as depth increased.

At approximately 4 to 8 WAT, reductions in sediment redox potential were noted in herbicide-treated flats (Table 3.5) These reductions coincided with the complete loss of the vegetative canopy and presumably the root system It should be noted that there was greater reduction in sediment redox in the sandy sediments compared to the potting soil-based sediments These results were not surprising as it is well known that roots of aquatic macrophytes can directly influence sediment redox, especially in sandy sediments with a low organic matter content (Carpenter et al 1983, Jaynes and Carpenter 1986) In these studies, hydrilla root systems were almost completely removed by 8 WAT following the herbicide treatments. It is likely that the inability to detect any changes in sediment redox in the potting soil as compared to the sand sediments following these herbicide treatments is due to the increased organic matter and higher reduction potential in the potting soil This rapid consumption of oxygen in organic sediments following its release from macrophyte roots has been noted by Jespersen et al (1998). Moreover, the fact that the vast majority of the hydrilla root system was concentrated at the bottom of

the containers increased the likelihood that any significant redox changes would likely occur in a limited area

There was a significant interaction ($p < 0.05$) between treatment and DAT Initial tuber sprouting following treatment was low, with no differences noted between any of the treatments thru 4 WAT From 8 thru 20 WAT, analyses indicated the sprouting response differed due to treatment effects (Figure 3 1) Increased sprouting in both the endothall and copper treatments as well as treatments covered by an artificial canopy were observed in both Studies 1 and 2 for plants grown in plastic flats In contrast, mechanical clipping treatments that resulted in canopy removal did not stimulate sprouting compared to untreated controls.

Data from studies 1 and 2 were not pooled due to differences observed in overall sprouting rates; however, both studies resulted in a significant stimulation of a sprouting response following both herbicide treatments and the artificial canopy treatments Differences in treatment dates (early July versus late May) between the studies may have contributed to some of the differences noted in the overall sprouting rates Recent reports by Madsen and Owens (1998) and data on tuber sprouting dynamics (Chapter 4) and disturbance (Chapter 5) suggest that dioecious tubers may be predisposed to sprout (especially when disturbed) in August thru October

Study 2 -Both Sediment and Container Type Impacted Tuber Sprouting

Sprouting response was influenced by sediment type, treatment regime, and time (main effects), however, no interaction between the effects was detected (Figure 3 2) Differences in the sprouting response due to sediment type were noted throughout the study, whereas, treatment effects were not significant until 8 WAT Tuber sprouting in

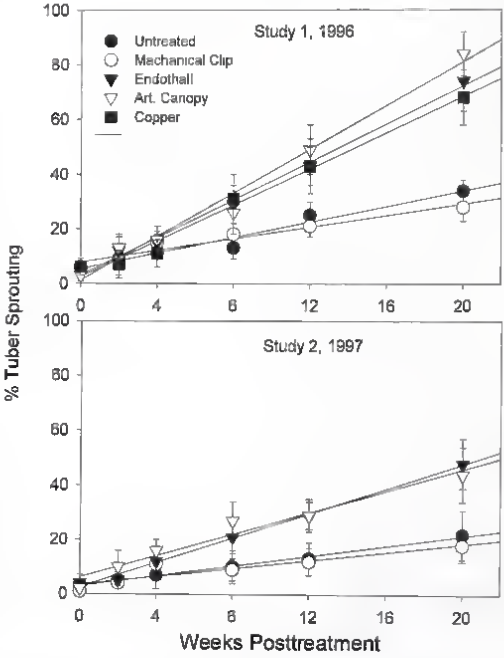

Figure 3 1 Mesocosm evaluation of the impact of various treatment methods on the sprouting of hydrilla tubers In study 1, hydrilla was planted in flats in September 1995, treatments were applied in July 1996 In study 2, hydrilla was planted in flats in August 1996 and treatments were applied in May 1997 Each data point (+ 1 SD) represents the value obtained in flats harvested in 3 replicate tanks

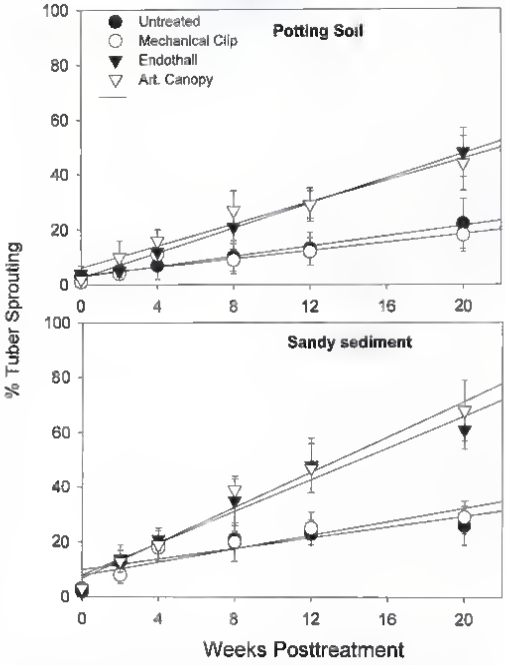

Figure 3 2 Mesocosm evaluation of the impact of various treatment methods on the sprouting of hydrilla tubers grown in potting soil or sand sediment in plastic flats Overall sprouting rates were significantly enhanced in the sand sediments compared to potting soil. Each data point (+ 1 SD) represents the value obtained in flats harvested in 3 replicate tanks.

sand sediments was greater than that observed in the potting soil As noted above, the sand sediments became more reduced following herbicide treatment, whereas, no differences in sediment redox were detected in any of the potting soil treatments Changes in reduction potential of the sand sediments suggest that hydrilla roots were contributing oxygen to the sediments prior to lethal treatment effects In contrast, the lack of redox change in the potting soil media is likely related to the fact that reduction potential is so high that oxygen is consumed immediately upon release from the hydrilla roots (Sand-Jensen et al. 1982, Jaynes and Carpenter 1986) Van and Haller (1979) have also demonstrated that following management, sprouting rates of tubers formed in sandy sediments were increased compared to sediments containing high clay or organic matter

Tuber sprouting was also affected by the container type, treatment, and time, however, a significant 3 way interaction was also indicated. One-way ANOVA at each harvest date indicated tubers in plastic containers significantly increased sprouting at weeks 8 through 20 following herbicide treatment, whereas, no treatment differences were noted in the PVC cores throughout the study (Figure 3 3) This lack of sprouting response in PVC cores was also noted and quantified in studies described in Chapter 2 (depth of formation and sprouting) Differential sprouting observed in pvc cores and plastic containers was unequivocal and suggests the choice of experimental materials can confound study results Nevertheless, the increased sprouting response following treatment of the plastic flats compared to pvc cores yields several lines of evidence to refute proposed hypotheses on factors influencing tuber sprouting. Results of these studies rule out several simple hypotheses that have been posited to explain increased sprouting. Moreover, evidence from these studies suggests that a close association

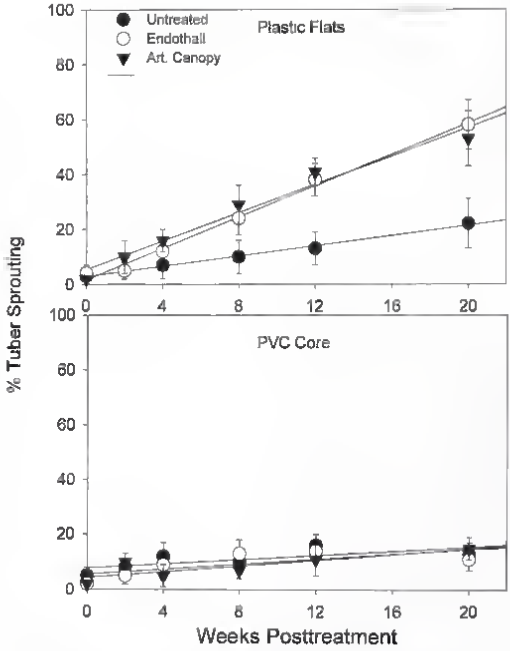

Figure 3 3- Mesocosm evaluation of the impact of three treatment methods on the sprouting of hydrilla tubers grown in shallow plastic flats containing potting soil or in 10 cm diameter x 30 cm tall pvc cores containing potting soil Differences between treatments were noted in plastic flats at greater than 8 WAT, whereas no sprouting differences were noted in the pvc cores throughout the study Each data point (+ 1 SD) represents the value in flats collected from 3 replicate tanks

between hydrilla roots and detached tubers may explain the increased sprouting rates observed in the plastic flats

Higher sediment temperatures and light penetration observed in the mechanically clipped treatments had no impact on tuber sprouting in any studies when compared to untreated controls. These results allow rejection of the simple hypothesis that removing a hydrilla canopy stimulates tuber sprouting. Furthermore, the fact that placement of an artificial canopy (sediment temperatures and light penetration were similar to untreated controls) did not inhibit sprouting of hydrilla tubers also allows rejection of the hypothesis that the mere presence of a canopy prevents sprouting of hydrilla tubers. The only factor common to treatments which stimulated tuber sprouting was the ability to kill both the shoot and root system of the hydrilla. Harvest of the untreated controls and mechanically clipped treatments revealed extensive rooting systems in close contact with tubers at the bottom of the plastic pans, whereas, herbicide treated plants (including those with the artificial canopy) contained few if any viable roots by 8 WAT. Based on pretreatment harvests, this close association between roots and tubers was common to all flats prior to herbicide treatment. In contrast to the flats, harvest of the untreated PVC cores did not suggest any aggregation of hydrilla roots and tubers.

The artificially close association between an extensive root system that concentrates along the bottom of the shallow plastic containers, and detached tubers that also form in this same area, likely influenced the strong sprouting response observed. In these studies, where a close association between roots and tubers exist, the death of the rooting system likely changes the redox potential in the vicinity of the tuber from a partially oxidized environment to a highly reduced environment. Loss of the rooting

system changes the microenvironment of the root channel from a mildly oxidized zone to an area that is anoxic. Despite repeated attempts, the ability to demonstrate and then replicate this change in sediment redox where roots and tubers coexist has been unsuccessful. Problems associated with the blind placement of redox probes was not overcome to allow sufficient explanation of changes. In addition, in highly reduced sediments, oxygen is consumed quite rapidly, and aside from placing a redox probe directly on a root channel, it is unlikely that redox changes could be measured.

While data from this study has not quantitatively confirmed that sprouting is related to changes in sediment redox, Spencer and Ksander (1997) have demonstrated an increased sprouting response by hydrilla tubers moved from hypoxic to anoxic conditions in the laboratory. Due to oxygen release by intact root systems, the destruction of hydrilla roots in these studies also likely resulted in the environment of many tubers changing from hypoxia to anoxia. It is likely that use of oxygen microelectrodes as described by Sorrell and Dromgoole (1987) may be necessary to measure microsite changes around the tuber, in order to quantitatively demonstrate the role that oxidation/reduction plays a role in the sprouting of tubers.

These mesocosm studies also provided serendipitous data on the potential for sprouted hydrilla tubers to establish under a plant canopy. In these studies, survival and establishment of sprouting tubers under the detached artificial canopy was extremely low. Light readings under the artificial canopy (data not shown) were similar to light measurements recorded at the sediment surface under hydrilla canopies (1 m water depth) in Orange Lake, FL, Lochloosa Lake, FL, and research ponds at the Austin Cary Forest, FL. Under the artificial canopy, all sprouted tubers remained etiolated throughout the

study, and none was observed to have grown to the height of the mesh Based on the estimated number of tubers that sprouted, versus the number of viable stems counted during any given harvest, it is likely that meristems were not surviving very long Twelve flats containing hydrilla tubers that had sprouted and emerged were marked with small flagging stakes and then observed 30 and 75 days later Data suggest that approximately 40% of these shoots were no longer present at 30 days and 88% had perished by 75 days under the low light environment of the artificial canopy

Study 3 - Tuber Age Did Not Influence Sprouting Potential

Pretreatment results indicate that tuber numbers significantly increased as the age of the flats increased (Table 3 6). While this result was expected, the magnitude of the increase was smaller than would have been predicted based on tuber production and subsequent sprouting rates measured during the first year Although shoot biomass was similar among all three age classes of plants, rootcrown density decreased as the age of the flats increased (Table 3 6) Decreased rootcrown density may partially explain the lower than expected tuber values in 3-year-old flats Rootcrown density has implications regarding production and distribution of tubers, and will be discussed in Chapter 4

As noted in the previous studies, differences in tuber sprouting existed between treatments within each age class, however, flats containing multiple age classes of tubers did not influence response to treatment (Figure 3 4) While these results do not provide data on the percentage of tubers from each age class that sprouted, they suggest that sprouting of 2- and 3-year old tubers was not greater than newly formed tubers While field populations likely contain tubers of various age classes These results suggest that the age of the tubers is unlikely to influence sprouting following management.

Table 3 6 Tuber number, shoot biomass, and rootcrown density measured in June 1998 for hydrilla that was established in plastic flats in August 1995, 1996, and 1997

Establishment Date	Shoot biomass g dry wt/ flat	Tuber #	Rootcrown Density
August 1995	168 (31)	225 (22)*	4 (3)*
August 1996	179 (25)	184 b (13)*	10 (5)*
August 1997	159 (26)	153 c (18)	21 (4)

* Significant differences noted between 1995 and 1996 data compared to the 1997 data according to t-tests$_{0.05}$ (n=4).

Study 4 – Short-term Drawdowns can Stimulate Tuber Sprouting

No differences were noted between overall tuber production (44 ± 11) in the three sediment types, however, there was a significant ($p<.05$) three-way interaction between drawdown period, sediment type, and harvest date on the sprouting of hydrilla tubers Tuber sprouting was not different between any of the treatments at 30 DAT (Table 3.7) Data collected at 60 DAT, indicated that while drawdowns significantly stimulated sprouting in excess of 80%, the length of the drawdown period was not an important factor in the potting soil and Austin Cary (sand) sediments (Table 3 7) In contrast, a linear relationship was noted (r^2= 89) between the length of the drawdown and increased sprouting rates in the organic sediment from Orange Lake

Redox potential increased significantly in the potting soil and Austin Cary sediments within 24 hours of removing cores from the water (Table 3 8) In contrast, reductions in the Orange lake sediments were not measured until nearly 5 days after the drawdown and these data tended to be highly variable compared to the other treatments.

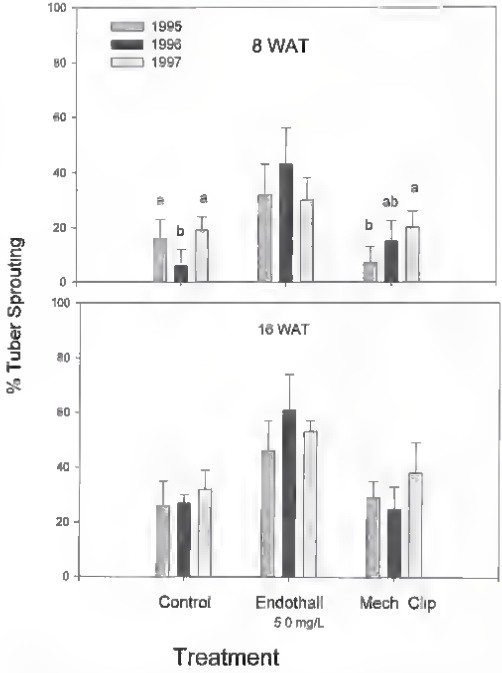

Figure 3.4 Mesocosm evaluation of the impact of 3 treatment strategies on the sprouting of hydrilla tubers. Flats established in 1995 contained tubers of 3 age classes, those established in 1996 contained 2 age classes, while those established in 1997 contained 1 age class of tubers. Letters above the bars indicate significant differences between treatments according to an LSD0.05 (n=3)

Table 3 7. Hydrilla tuber sprouting response following various drawdown periods

Drawdown (organic) (Days)	Sediment Source: Potting soil	Austin Cary (sand)	Orange Lake
	% Tuber Sprouting 30 DAT		
0	6	8	5
1	11	12	8
3	5	7	4
7	7	9	8
14	11	13	7
	% Tuber Sprouting 60 DAT		
0	12	9	11
1	88*	83*	17
3	85*	81*	26*
7	81*	79*	39*
14	92*	85*	68*

*Sprouting values are significantly different from untreated controls according to Dunnett's test$_{0.05}$(n=6)

Table 3 8 Sediment Redox potential (mV) measured at an 8 cm depth in 3 sediment types prior to a drawdown, during a 14-d drawdown, and at 24 and 48 hr following re-flooding. Measurements were taken in 10 cm diameter PVC cores that had been planted with hydrilla.

	Sediment Source		
Days After (organic) Drawdown	Potting soil	Austin Cary (sand)	Orange Lake
Untreated	-311*	-249	-344
Pretreatment	-308	-257	-357
0	-297	-244	-349
1	-82*	-122*	-337
2	29*	-15*	-294*
5	159*	87*	-113*
7	121*	111*	-297
10	177*	131*	-95*
14	133*	125*	-240*
1 (re-submerged)	-245*	-78*	-324
2 (re submerged)	-315	-217	-330

*Values within each sediment type are different from untreated controls according to Dunnett's test$_{0.05}$ (n=5)
*All data is reported for June 1997

It is suspected that frequent thunderstorms confounded redox data in the slow-draining organic cores Moreover, these rains prevented collection of meaningful soil moisture content data

Regulating water levels in the mesocosms at 15 cm resulted in rapid drying of hydrilla shoot biomass, and subsequent seed germination of several terrestrial species (dominated by sedges) in the pvc cores. Sprouting data collected at the 30 day harvest indicated that sprouting was not immediately influenced and remained at less than 10%. Unexpectedly, sprouting rates exceeded 70% by the 60 day harvest (Table 3 7) Based on the first drawdown study, it had been hypothesized that re-flooding following a drawdown acted as a stimulus (possibly by altering redox) to increase sprouting rates Due to the wicking action in the partially-flooded mesocosms, soils remained quite moist throughout the study, thereby allowing meristems of the sprouting tubers to survive just below the soil-air interface Observations suggested the meristems were viable and would have rapidly grown upon re-submerging the cores Spencer and Kaander (1992) have reported that tubers of *Potamogeton* species can sprout during drawdown conditions under favorable sediment moisture and temperature regimes These results further support the contention of Sculthorpe (1967), that hydrilla evolved in a monsoonal climate, and tubers are stimulated to sprout by the flooding rains.

Comparison of sprouting rates of tubers in the upper 15 cm of the cores versus those in the lower 15 cm showed sprouting rates differed significantly (Table3 9) Observations at the 70-d harvest indicated that partitioning the cores into intervals smaller than 15 cm may have improved interpretation of the data. It was noted that the majority of quiescent tubers found in cores containing a barrier at 15 cm, were found near

Table 3 9 Sprouting of hydrilla tubers in 30 cm tall pvc cores that were placed in water at a depth of 15 cm

Treatment	Tuber #	%sprout at 30 DAT	% sprout at 70 DAT
Barrier at 15 cm			
Upper 15 cm	50*	8	72
Lower 15 cm	3	na	na
No Barrier			
Upper 15 cm	39*	7	77*
Lower 15 cm	21	4	13

*Values indicate differences between treatments according to a t-test$_{0.05}$ (n= 10)

the barrier Although a limited number of tubers formed at depths less than 8 cm, nearly 100% of these tubers had sprouted.

Redox data were quite variable and therefore use of this data to explain the sprouting responses observed was not attempted Probes placed at the 18 cm depths (continuous flooding) remained reduced throughout the study and are in agreement with results observed in previous studies In contrast, readings taken at the 10 cm depth ranged from suggesting the sediments were becoming somewhat oxidized to readings that were no different from submerged cores While readings within each core remained fairly consistent over time, the variability between cores (often exceeding differences of 250 mv) made this data difficult to interpret This was especially true in light of the fact that the sprouting response observed was fairly consistent regardless of redox readings in a given core

Summary and Conclusions

Results of these studies refute earlier hypotheses which suggested that removal of a vegetative canopy of hydrilla stimulated tuber sprouting due to increased sediment temperatures or light penetration. Differences in tuber sprouting for plants treated in July compared to May suggest that dioecious tubers may be predisposed to sprout in the late summer and early fall following a disturbance. Although direct empirical evidence was difficult to obtain, it is hypothesized that a close association between detached tubers and hydrilla roots in shallow plastic flats influences tuber sprouting. Following management techniques which resulted in death of the root system, sprouting rates greatly increased compared to tubers which remained in flats with an intact functioning root system. Moreover, in containers which prevented a close association between roots and tubers, management (resulting in root death) did not stimulate sprouting. These results suggest that earlier research conducted in these shallow flats may have provided confounding information concerning management impacts on sprouting of hydrilla tubers. While these studies did not provide quantitative evidence of an association between sediment redox change and increased tuber sprouting, the close association of roots and tubers in flats, increased sprouting in sand sediments (where plant roots have a greater impact on sediment redox), and the general lack of increased sprouting response in experimental units (PVC cores) that prevented association of roots and tubers, all strongly suggest a role for changes in sediment redox influencing sprouting of *in situ* tubers. Measurement of changes at the microsite level will require more sophisticated analyses and experimental design. Treatment of various age classes of tubers suggested that tuber age does not influence the likelihood of sprouting following various management techniques

Short-term drawdowns (24 to 48 hours) resulted in a notable increase in tuber sprouting. Previously, it had been hypothesized that a long-term (months) drawdown was necessary to condition the tuber for sprouting

CHAPTER 4
MANAGEMENT IMPACTS ON DIOECIOUS HYDRILLA TUBER SPROUTING AND TUBER POPULATION DYNAMICS

Introduction

Among nuisance macrophytes, hydrilla has been particularly difficult to manage, due in large part to the unpredictable nature of a substantial underground population of tubers. For example, Lake Lochloosa near Gainesville, Florida was treated with the herbicide fluridone in 1993 and 1994 resulting in near complete control of hydrilla. Hydrilla had become limited to just a few fringe areas around the lake in 1995 and 1996 and then in the late summer and fall of 1997, numerous individual clumps of hydrilla were noted throughout the whole lake. The sudden emergence of isolated clumps of hydrilla suggested the possibility that tuber sprouting may likely have been stimulated. An increase in water clarity coincided with the increase in hydrilla, however, it is unclear if improved water clarity led to increased hydrilla growth and establishment or if increased hydrilla growth led to clearing of the water. Furthermore, the role of tubers in the increase of hydrilla coverage was not documented, and therefore a primary role by fragments cannot be dismissed. Sampling difficulties in the field associated with the clumped, non-random distribution of tubers (Haller and Shireman 1983, Sutton and Portier 1985, Spencer et al. 1994b), has generally precluded data collection to support or refute the hypothesis that a change in environmental conditions (i.e. an external factor) stimulates sprouting of hydrilla tubers.

One explanation for the sudden lake-wide emergence of hydrilla is an increased probability of establishment of newly sprouting tubers. This hypothesis would suggest that tubers sprout in a fairly random and uniform manner, and it is the current prevailing conditions (light penetration, herbivory, temperature, etc) that dictate whether or not establishment is likely. This hypothesis is supported by the work of Sutton and Portier (1985) who reported that tuber sprouting was erratic and non-seasonal in South Florida.

While rapid re-infestation of treatment areas is often attributed to sprouting tubers (this claim is generally unsubstantiated), it is their potential for long-term survival (4 years and greater), and their somewhat erratic sprouting characteristics that make decisions concerning hydrilla management particularly difficult. Again, Lake Loshloosa, FL provides an example of the difficulties involved in prioritizing management of hydrilla. Following the emergence of hydrilla in the late summer of 1997, plans were made to conduct further treatments, however, no treatments were conducted in the Fall and high water flow due to the extremely heavy rains in the winter of 1998, precluded fluridone treatments. While the high water greatly reduced hydrilla biomass, the lack of scheduled treatments in the fall allowed new tubers to be formed, essentially insuring that a substantial population of hydrilla propagules will remain present in this lake for the next 3-5 years. As the State of Florida is currently engaged in a large-scale effort to bring hydrilla under maintenance control, the behavior of tubers will ultimately determine the success or failure of this program.

Although research on sprouting of hydrilla tubers has certainly not been neglected, the vast majority of investigators have chosen to remove tubers from their *in situ* environment to study them. This change in environment generally stimulates a rapid

sprouting response (often in excess of 90% within 7 d) suggesting that the tubers are not dormant, but under an environmentally imposed quiescence (Van and Steward 1990) Laboratory sprouting studies have provided valuable information, yet they have done little to explain factors that influence *in situ* sprouting of hydrilla tubers To date, very few authors have attempted to characterize *in situ* tuber sprouting and long term tuber population dynamics Sutton and Portier (1985) have provided the most comprehensive data for several non managed lakes in South Florida, and Sutton (1996) has reported on an intensively managed canal in South Florida. In addition, Haller and Shireman (1983) characterized tuber sprouting following successive drawdowns on a North Florida Lake, and Steward (1980) provided quantitative data on tuber populations in ponds 14 months after they were treated with the long residual herbicides fenac and dichlobenil

Literature from terrestrial disciplines documenting the population dynamics of perennial species is relatively scarce, with most of the focus placed on seeds and seed dormancy The lack of information on hydrilla tuber population dynamics following management as well as the fact that terrestrial studies do not provide a good model system which describes the behavior of hydrilla tubers, suggests research in this area would improve our understanding of the population dynamics of tubers and allow for improved hydrilla control strategies

Research by Sutton and Portier (1985), suggests that in non-managed areas, hydrilla tuber sprouting varied both temporally and spatially...and that a low percentage of sprouting tubers was observed in *in situ* populations Due to sampling variability reported tuber densities often varied considerably within a sample site however, in these lakes dominated by hydrilla, the tuber populations did not generally increase over time

This observation is perplexing because the low sprouting numbers would suggest that either a substantial proportion of tubers perishes each year, or tuber production becomes greatly limited over time. While increasing water depth has been shown to reduce tuber production (Miller et al 1976, McFarland and Barko 1995), to date there are few hypotheses to explain why tuber populations are thought to remain relatively stable over time.

One chemical control strategy that has recently been suggested to impact tuber populations is the application of the systemic herbicide fluridone. Long-term efficacy following fluridone application in some Florida Lakes has led to speculation that these treatments may be impacting tuber populations by stimulating sprouting. Lending credence to this hypothesis is the fact that fluridone affects abscisic acid (ABA) biosynthesis and it is often used in physiology labs in seed dormancy research (Rock and Quatrano 1995). The role of fluridone in disrupting tuber production by preventing synthesis of ABA has been established (MacDonald 1993), however, the potential role of fluridone in promoting the sprouting of quiescent tubers has not been determined.

To date, the population dynamics and sprouting response of hydrilla tubers following various management methods have been poorly characterized. The objective of this study was to intensively monitor the long-term response of tubers in the field following various management techniques. These studies also provided an opportunity to test hypotheses on factors that impact long-term tuber production, response of *in situ* tubers to fluridone treatment, and comparison of results between field and mesocosm studies described in Chapter 3

Materials and Methods

Site Selection and Description

A series of seven ponds located at the Austin Cary Forest in Gainesville, FL were chosen to evaluate hydrilla tuber population dynamics and sprouting response following various forms of management. These ponds were approximately 40 m long x 20 m wide x 1.2 m deep and each pond was plumbed to allow well water to be added to maintain water levels. Criteria used to choose these ponds as research sites included the fact that these ponds have contained hydrilla since their construction in 1974, and therefore represented one of the oldest continuous populations of hydrilla in the country. The long-term presence of hydrilla in shallow ponds had allowed a considerable tuber bank to form. Sampling prior to choosing these ponds as research sites suggested that in comparison to reports from field studies, these ponds contained a fairly uniform distribution of tubers, as well as densities that were much greater than are generally reported in the literature. The high densities and relatively low sample variation observed in these ponds was viewed as a significant improvement over the clumped and non-random distributions often observed under field conditions (Haller and Shireman 1983, Sutton and Portier 1985, and Spencer et al. 1994b). In addition, the fact that these hydrilla infested ponds were uniform in size and under the same environmental conditions allowed for replication of treatments.

Pretreatment sediment sampling in some of the ponds revealed that while most samples consisted of a high sand fraction (70%) and low organic matter content (<4%), locations within some ponds were characterized by areas with high organic matter content (>70%), or a high clay fraction (>50%). Infringement of cattails and torpedo grass in

localized areas of ponds was generally correlated with increased organic content of the sediments. Pond 1, pond 3 and pond 4 each contained areas with either high organic matter content or high clay content. These areas were delineated to allow for future comparison of sprouting rates between sediment types within each treated pond. In order to compare sprouting in different sediment types, 15 core samples were collected from each sediment at 2, 4, 6, 12, 18, and 24 months posttreatment. Sprouting data were subjected to ANOVA.

Population Dynamics and Sprouting Response

In April 1996, seven ponds containing moderate to dense hydrilla cover were selected and pretreatment data were collected. Shoot biomass was measured in each pond by randomly tossing a 1/4 m^2 frame into each of four sampling quadrants established in the ponds. Four samples were collected in each quadrant and shoots within the frame were harvested and dried to a constant weight at 70 C for 48 hr.

Pretreatment tuber values were determined by randomly collecting 10 sediment cores in each quadrant (40 samples per pond). A stainless steel coring device (10 cm diameter) was used to collect intact cores to a depth of 20 cm. Initial depth distribution of tubers in the Austin Cary ponds is well-characterized in Chapter 2. The total number of tubers within each core was quantified, as well as their physiological status (sprouting, rotting, quiescent). In addition, in May of 1996, 1997, and 1998 all tubers collected within each pond were classified according to size distribution based on their fresh weight. Size classes included 0-50, 50-150, 151-250, 251-350, 351-450 and > 450 mg.

Prior to treatment, redox probes (described in Chapters 2 and 3) were placed in three quadrants of each pond at sediment depths of 0, 2, 6, and 12 cm. To determine

sediment redox potential, platinum electrodes, specially constructed for field use, were inserted into the sediment and redox readings were taken using a hand-held pH/mv meter with a calomel reference electrode and adjusted to the hydrogen electrode standard by adding 224 mv. Hydrolab® datasondes were also deployed in ponds to record temperature and dissolved oxygen levels at 6 hr intervals near the sediment water interface. Irradiance was measured with a Biospherical QSL100 (quantum scalar irradiance meter) using a submersible probe.

Following pretreatment data collection, ponds were treated on May 19, 1996 with contact herbicides (endothall, diquat + chelated copper), a systemic herbicide (fluridone), or grass carp (Table 4.1). As described in the mesocosm studies, ponds treated with contact herbicides were treated at high rates to ensure rapid removal of the surface canopy. These ponds were re-treated at 4 weeks to remove any plants that may not have been injured following the first treatment. The grass carp pond contained >5 carp over 10 Kg in weight, virtually ensuring that no vegetation would establish during this study.

Following herbicide treatment, a total of 40 core samples (10 from each quadrant) were randomly collected in each pond on a monthly basis through December 1997, and every other month through November 1998. Vertical distribution of all tubers as well as vertical position of sprouting tubers was quantified through August 1997. Size classification of tubers was determined at 0, 6, 12, 18, 24, and 30 months after treatment. Following collection of tubers from each pond, the % viability was determined by placing the tubers in petri dishes containing distilled water for a period of 14 days. At this time the number sprouting, rotting, and quiescent were determined.

Based on observed differences in sediment types in large localized areas of 3 of

the 7 ponds, 15 core samples were collected from each sediment type (sand, clay, organic) to determine if differences in sprouting response could be detected. These samples were collected at 2, 4, 6, 12, 18 and 24 months after treatment.

In order to determine the potential of sprouting tubers to re-infest the ponds following various treatments, shoot biomass was measured in each pond in 3, 6, 9, 13, 15, 18, 20, 25, 27, and 30 months after treatment. Routine applications of contact herbicides were applied to specified ponds in August 1996 January 1997, August 1997, and January 1998 to ensure no new tubers were produced. August treatments were conducted to control a substantial amount of biomass that resulted from sprouting tubers over summer, whereas February treatments were considered to be more prophylactic to prevent a small number of newly sprouted tubers from forming new tubers The pond treated with the liquid formulation of fluridone required additional treatment in June 1997 and June 1998 to control substantial regrowth from tubers, whereas ponds treated with the granular formulation of fluridone received a prophylactic treatment in August 1998 (Table 4 1)

Tuber Production Following Biomass Recovery

Three ponds (endothall-treated, liquid fluridone-treated, and grass carp) were allowed to become re-infested with hydrilla in the summer of 1998, in order to determine how rapidly tuber populations can reestablish to the baseline numbers measured in the spring of 1996 In June, August and November of 1998, a 1/4 m^2 PVC frame was randomly placed in the ponds to determine the rootcrown density Ten samples were collected in each quadrant of the ponds Core samples in all ponds were collected in November 1998, however newly formed tubers were identified by their attachment to rhizomes or continued presence of stem tissue

Table 4 1 Treatment rates and schedules used to control hydrilla in ponds located at the Austin Cary Forest

Treatment	Rate, mg/L	Treatment Schedule
Untreated		
Untreated Pond 5	0 0	
Untreated Pond 7	0 0	
Contact Herbicide		
Diquat + Cu Pond 1	2 0 + 1 0	5/96, 6/96 8/96 1/97, 8/97, 1/98, 6/98 8/98
Endothall Pond 4	5.0	5/96, 6/96, 8/96, 1/97, 8/97, 1/98,
Systemic Herbicide		
Fluridone, liquid Pond 3	0 09	5/96, 8/97
Fluridone, granule Pond 2	0 09	5/96, 6/98
Grass Carp Pond 6	>125 / acre*	5/96*

*Rate reported as the estimated number of fish per vegetated acre
*Grass carp were present long before initiation of the study, but it is suspected that there was a weather-related fish kill (due to excessive heat and drought and ash from nearby forest fires) during May July 1998

near the base of the tuber as well as the characteristic purple color (attributed to anthocyanin) noted at the apex of newly formed tubers

Fluridone Pore water Concentrations

Fluridone concentrations were monitored in the water column on a weekly basis through 6 months posttreatment As aqueous concentrations began to approach <1 µg/L, it was noted that sprouted tubers were capable of establishing in the ponds treated with the liquid formulation, whereas, no sign of sprouted tubers was noted in ponds treated with the slow-release granular formulation It was hypothesized that fluridone may be present near the sediment-water interface, thereby disrupting hydrilla growth from sprouting tubers In order to improve sampling techniques for fluridone and to test the hypothesis that fluridone was present near the sediment-water interface, pore-water equilibrators (Hesslein 1976), commonly called peepers, were constructed for this study using 2 cm thick plexiglass standing 30 cm in height, and containing twelve 20 mL sampling wells at 2 cm intervals Peepers have typically been used for determining concentrations of N and P in sediment pore water to determine fertility of sediments Distilled water was sparged with nitrogen for 1 hour to remove oxygen. The nitrogen-sparged water was carefully poured into the peeper wells and a 2 micron nucleopore polycarbonate membrane (Corning) was placed over the wells The protective plexiglass face-plate was secured and protects the membrane from tearing. The peepers were immediately deployed in the ponds so that 6 wells (12 cm) were below the sediment surface, one well was at the theoretical sediment-water interface and 5 wells were above the sediment (10 cm)

Based on initial laboratory studies peepers equilibrated with external aqueous

fluridone concentrations within a 48-hr period Nonetheless, peepers deployed in the field were allowed to equilibrate for a 14 to 21 day period. Upon removal from the water, a syringe was inserted into a designated peeper well and the 20 ml sample volume was extracted and placed in a 60 ml amber polyethylene bottle Samples were analyzed using both traditional high performance liquid chromatography (HPLC) and a new enzyme-linked immunoassay technique provided by the SePRO Corporation. Samples were collected at 8, 12, 16, and 20 months posttreatment.

Results and Discussion

Following the May 1996 treatment with contact herbicides, hydrilla was severely injured and biomass decreased significantly by 6 WAT (Figure 4 1) Although excellent initial results were observed, these ponds were retreated at 4 weeks to ensure that any vegetation other than tubers (or axillary turions) was completely removed from the ponds Regrowth from sprouting tubers required that several additional treatments be conducted to prevent formation of new tubers (Table 4 1) In contrast to the rapid action of the contact herbicides, treatment with the systemic herbicide fluridone resulted in slow onset of injury symptoms and very little reduction in biomass until 12 to 16 WAT Following fluridone treatment no regrowth was noted through 52 WAT Grass carp ponds had little vegetation at the beginning of the study and remained void of hydrilla through 107 WAT Grass carp and diquat/copper-treated ponds generally remained devoid of any vegetation, whereas endothall and fluridone-treated ponds were often rapidly colonized by *Chara* Untreated ponds maintained relatively consistent levels of biomass throughout the year

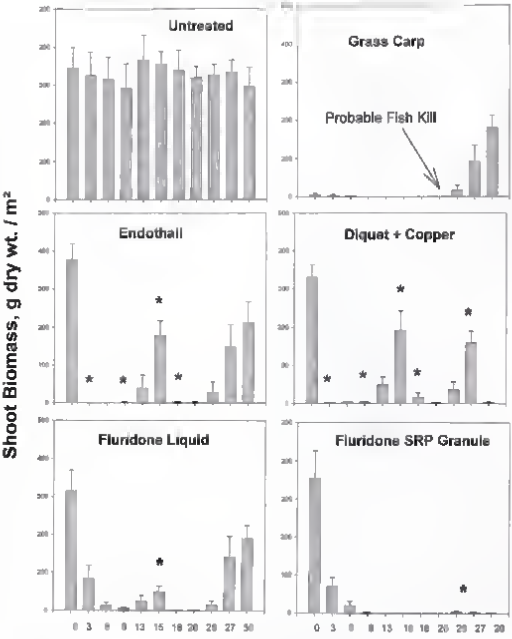

Figure 4 1 Hydrilla shoot biomass measured in the Austin Cary Research Ponds following various management techniques Initial herbicide treatments were applied May 19, 1996 (time 0) Each bar represents the average of 9 samples collected with a 1/4 m² PVC frame Asterisks above the bars represent times when the ponds were retreated to prevent hydrilla from forming new tubers.

Tuber sprouting remained between 0 5 and 7 percent of the total population at any given sample period and was similar among all ponds throughout the study (Figure 4.2) A slight peak in sprouting rates was noted in all ponds during the October thru December sampling periods during 1996, 1997, and 1998 Madsen and Owens (1998) have recently reported a sprouting peak for dioecious hydrilla tubers in August Based on results of studies in Texas and Florida, dioecious tubers may be predisposed to sprout in the late summer or fall It has recently been suggested that this late summer sprouting could be related to a large number of heating-degree days (D Spencer personal communication) Nonetheless, it should be noted that the early Fall sprouting peaks noted in Florida still only represented <7 percent of the total tuber bank

Overall these results indicate that management had no effect on tuber sprouting. During several sampling times, the visual appearance of the treated ponds suggested that tuber sprouting was prolific as apices of hydrilla were abundant just above the sediment surface. Nonetheless subsequent sampling revealed that percent tuber sprouting was an exceedingly low percentage of the total tuber population Based on these results, mesocosm studies using shallow flats which allowed for close association of tubers and roots (Chapter 3) were not predictive of tuber sprouting in the ponds In contrast, mesocosm studies using potting vessels (30 cm tall PVC cylinders) that prevented this close association of tubers and roots were predictive of field results obtained in this study

In previous mesocosm studies, sprouted tubers were observed to remain viable in the sediment for up to 2-4 months prior to emerging (Chapter 2). This observation is supported by laboratory work from Spencer and Ksander (1996) and Bowes et al (1979) who report that a sprouted tuber can survive on carbohydrate reserves for up to 3 months

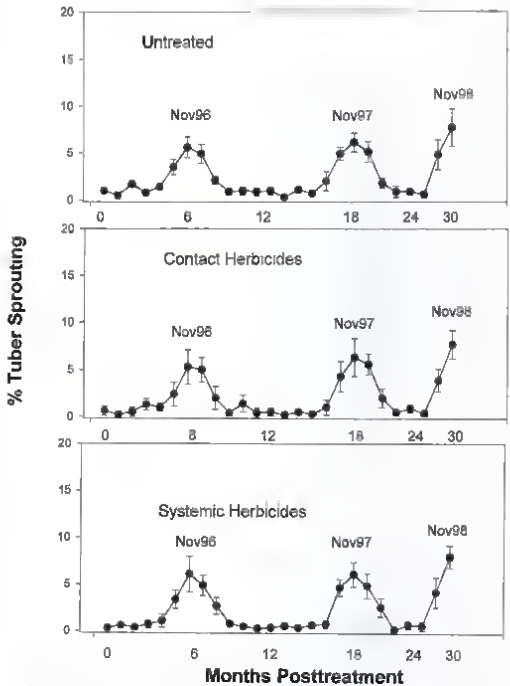

Figure 4-2. Percent of tubers sprouting following several management techniques applied to control hydrilla in small research ponds at the Austin Cary Forest, FL. Contact herbicides are combined results of endothall and diquat/copper, and systemic herbicides include both fluridone SRP and AS ponds. Grass carp ponds displayed a similar sprouting response (data not shown). Each point represents the average of 80 (+ 1 SE) core samples from two ponds.

It had previously been suspected that a sprouted tuber had to emerge rapidly in order to survive, however if tubers emerge slowly in the field, the results can be somewhat confounding in terms of predicting sprouting peaks For example, a tuber that had sprouted in September might not be collected until December yet it would be designated as having sprouted in December The fact that no differences existed between treatments in this study reduces the that likelihood that this problem led to any misinterpretation of the data. Nonetheless, it is likely that a significant fraction of the sprouted tubers collected in November had sprouted in September through October The unexpected sprouting peaks noted in November (often carrying through January) when water temperatures are generally below optimum for sprouting of tubers is likely influenced by the fact that sprouted tubers often don't rapidly emerge. The role of how cooling water temperatures affected emergence of sprouted tubers was not addressed in this study, but it is likely that elongation is slowed in the cooler waters

Sprouting was again shown to be unrelated to depth of the tuber in the sediment in any of the treatments; however, during subsequent analysis of the data, it was observed that 57% of samples from a depth profile that yielded a sprouting tuber had more than 1 tuber sprouting. The lack of a differential sprouting response with increased depth in the sediment suggested that there was no pronounced gradient from 2 to 16 cm with respect to critical environmental factors that influence sprouting The fact that sprouting tubers were such a low percentage of the total population (<3% at most times), indicating that the high incidence of multiple sprouting was not a random occurrence. These data support the hypothesis by Nesser et al (1997) on purple nutsedge (*Cyperus rotundus*), that variations at the microsite level likely influence tuber sprouting

No differences in percent sprouting rates were noted in the sand, clay, and organic dominated substrates throughout the study (Figure 4 3) Although tuber densities tended to be lower in areas high in organic matter, this was attributed to prior competition from *Typha latifolia* and *Panicum repens* which prevented dense establishment of hydrilla in these areas. While it has been observed that tuber sprouting in organic sediments following a drawdown is often significantly lower than that observed in sand sediments (Haller and Shireman 1983, Chapter 2), results from this study suggest that sprouting remained low in all sediments tested. It should be noted that redox potential in all sediment types indicated highly reduced conditions throughout the study regardless of treatment regime

Although tuber sprouting remained quite low throughout the study, it was observed that following treatment, greater than 35% of the axillary turions collected the following Spring in 1997 (March-May) were sprouting in the treated ponds In contrast, only 9% of the axillary turions collected in the untreated ponds were observed to sprout during this period It should be noted that axillary turion populations were only 4% of the tuber population and therefore while sprouting of axillary turions was greatly increased, it did not represent a significant increase in total sprouting compared to the tuber population Axillary turions became very scarce in the treated ponds from August of 1997 through the remainder of the study The short-term nature of the axillary turion as a survival structure has been described by both Spencer and Rejmanek 1989 and Van and Steward 1990. Nevertheless, increased sprouting of axillary turions suggest that changes in the environment near the sediment surface (increased light, DO, or temperature) stimulated sprouting in comparison to those turions which remained under a canopy

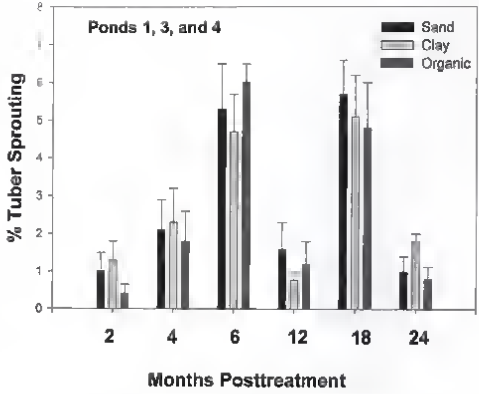

Figure 4-3 Hydrilla tuber sprouting in 3 sediment types characterized in research ponds at the Austin Cary Forest FL Each bar represents the average of 15 samples (+ 1 SD)

Tuber populations in all treated ponds displayed a similar reduction over time that could be described by a linear function (Figure 4 4) While some authors have argued that modified exponential decay functions are more biologically realistic in terms of explaining propagule decline (Neeser et al 1997), the fact that greater than 50% of the original estimated population remained at 30 months after treatment allowed for linear treatment of the data. The viability of tubers removed from *in situ* conditions exceeded 90% in laboratory sprouting tests conducted throughout the study It was noted that tubers removed from the sediments in December thru February often displayed a delay in their sprouting response (2 to 4 weeks) compared to the rapid response (5-7 days) of tubers removed in the spring summer, and fall Results suggest that tuber viability was not influenced by their age, and based on laboratory viability tests the vast majority of that remained as part of the the *in situ* tuber population maintained the potential to reinfest the ponds at 30 months posttreatment.

Tuber size did not influence the likelihood of sprouting either *in situ* or in the laboratory viability studies. While the ability of propagules of various size classes to became established following sprouting was not documented in this study, it has been suggested that propagule size is key to the establishment and survival of hydrilla tubers (Spencer 1987, and Spencer et al 1987)

The size distribution of tubers in the treated ponds changed over time, as the smallest class of tubers became less abundant within 1 year posttreatment (Figure 4 5) The sprouting data did not demonstrate an increased likelihood of smaller tuber sprouting, therefore it was presumed that this decrease in smaller tubers was due to

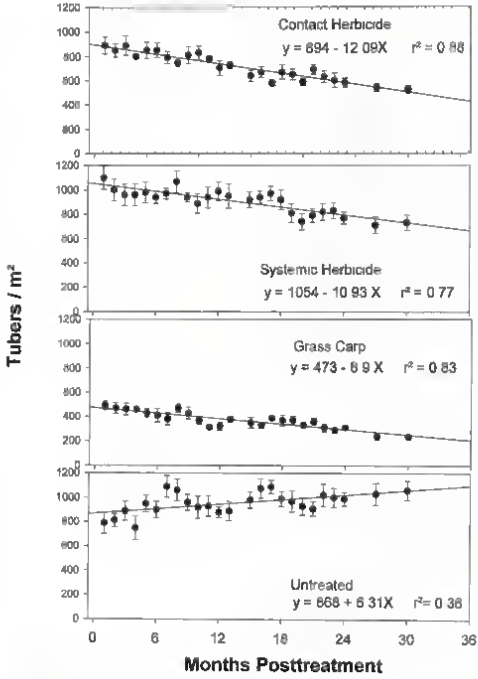

Figure 4 4 Regression analysis of hydrilla tuber populations measured over a 30 month period following intense management in research ponds at the Austin Cary Forest, FL Each data point represents the mean of 80 (+ 1 SE) core samples (the grass carp pond data contains only 40 samples for each data point)

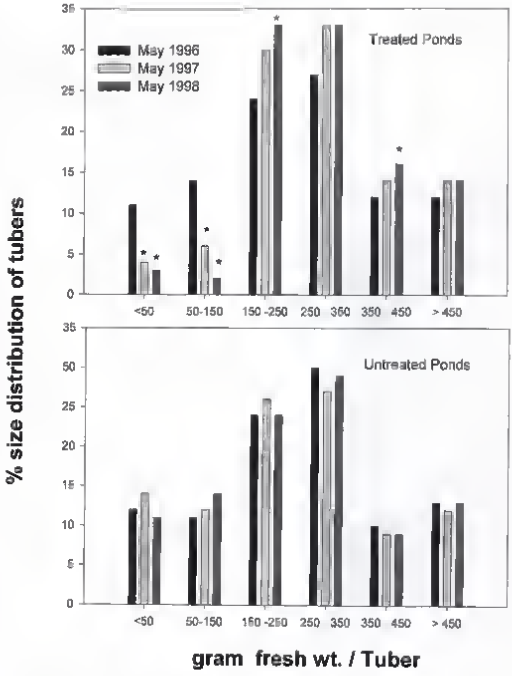

Figure 4 5 Size distribution of hydrilla tubers over time, in research ponds at the Austin Cary Forest, FL that were intensively managed compared to untreated ponds Each bar represents the mean of 40 samples Asterisks above the bars indicate differences between treated and untreated ponds according to a t test@ 05

increased mortality Based on Spencer's observations noted above, the loss of these small tubers may not be particularly relevant, because smaller tubers are less likely to become established. Although overall tuber mortality would be expected to increase with age due to a gradual loss of physiological integrity, only a small but consistent proportion of rotten tubers (0 1 to 0 4%) were observed during sample processing. As noted by Haller and Sutremon (1983), it remained unclear if these rotten tubers had sprouted and become detached from their apical shoot or if they had simply perished While there has been some speculation that tubers are susceptible to fungal attack (Berhardt and Dunlway 1986), it should be noted that in the anaerobic environment inhabited by tubers, fungal attack would be unlikely due to the fact that fungi are obligate aerobes. Personal observations from mesocosm studies conducted during this research do suggest that sediments in which sulfides (hydrogen sulfide) were noticeable due to their noxious odor were particularly detrimental to the survival of *in situ* tubers Sediments with notable sulfide odor often resulted in greater than 85% tuber mortality The buildup of sulfides and methane are well-described phenomena known to limit growth or exhibit toxic properties to many wetland and aquatic species (Koch et al 1990, Jespersen et al 1998).

In contrast to the treated ponds, untreated ponds resulted in a slight increase in tuber populations over time (Figure 4 4) This increase was not as well described by a linear function, likely due to the fact that values increase in the fall and spring and then slightly decreased through the summer While the overall increase in tubers was not surprising, the magnitude of the increase was much less than expected given the fact that hydrilla maintained a thick canopy throughout the study The fact that sprouting rates generally represented less than 4% of the total population, and mortality (i.e rotten

tubers) was rarely observed, led to speculation that an alternate factor may be limiting tuber populations. Based on long-term mesocosm studies and observations in the ponds, it was hypothesized that low rootcrown density may have been limiting the production of tubers in unmanaged, dense hydrilla.

Redox, temperature, and light data were similar to data collected in mesocosm studies (Table 4.2). Although redox readings tended to vary from site to site within and between ponds, following treatment, no significant changes in redox were noted at individual sampling sites throughout the study. In addition, although depth of placement of the redox probes (2, 6, 10 cm) within a sampling site often resulted in variable redox readings, readings at each depth remained constant following treatment. Overall, redox values ranged from -50 to -400 mV indicating that the sediments in these ponds were highly poised (i.e. very little fluctuation noted over time) and remained reduced regardless of treatment. Treatments resulting in removal of the surface canopy of hydrilla resulted in increased dissolved oxygen (DO) readings near the sediment/water interface. While DO readings at the sediment/water interface never exceeded 0.7 mg/L in the untreated ponds, values in treated ponds ranged from 0.5 to 3 mg/L. Although DO increased at the sediment/water interface, increased penetration of oxygen into the sediments was not indicated as redox readings at 2 cm sediment depths remained essentially unchanged. Following removal of the vegetative canopy, sediment temperatures increased 3 to 9 C above temperatures measured in the untreated ponds. Temperatures recorded 5 cm below the surface canopy of untreated ponds often exceeded 40 C while sediment temperatures remained near 22 ± 2 C. In contrast, removal of the vegetation in the shallow ponds resulted in fairly isothermal conditions from the surface

water to the sediments. These results suggest that the canopy serves as a heat trap during the day

Light readings measured at the sediment surface never exceeded 40 µmol/m²/sec in the untreated ponds Based on results of the mesocosm studies, the low light conditions that persisted under the canopy, likely prevented newly sprouted tubers from becoming established In contrast to the untreated ponds, light values measured at the sediment surface dramatically increased following removal of the vegetative canopy (Table 4 2) Light data recorded in the treated ponds often varied due to differences in water quality and an abundance of prostrate growth forms of *Chara* spp noted in several of the ponds Although there was a fair amount of variability between treated ponds, light reaching the sediment surface was generally at least 2 to 10 times that recorded in the untreated ponds

Aside from suggesting that increased light penetration, oxygen content near the sediment-water interface, or increased sediment temperatures stimulated axillary turion sprouting, these data did not provide any supporting information to explain sprouting of *in situ* tubers Based on data from the current study and mesocosm studies (Chapter 3) light and temperature can be ruled out as factors that significantly stimulate *in situ* sprouting of hydrilla tubers in North Florida. While there are likely age-dependent internal factors that regulate sprouting of vegetative propagules (Neeser et al 1997), the likely influence of environmental variations at the microsite level are suggested by results of both mesocosm and the current pond studies.

Table 4 2 Selected redox, temperature, dissolved oxygen, and light readings recorded in ponds at the Austin Cary Forest following various treatments

	Treatment Regime			
	Untreated	**Contact Herb**	**Systemic Herb**	**Grass Carp**
Redox, 6 cm (mv)				
6 WAT	- 240	-200	-195	-305
20 WAT	-190	-220	-170	-270
52 WAT	210	215	-182	-272
70 WAT	-221	211	-199	-295
104 WAT	-188	-223	-190	-315
Temp (C)				
6 WAT	22	28	22	29
20 WAT	21	29	23	29
52 WAT	21	27	28	28
70 WAT	23	29	31	31
104 WAT	22	28	29	29
DO (mg/L)				
6 WAT	0.5	0 1	0 5	1 4
20 WAT	0 3	1 9	0.2	2 4
52 WAT	0 5	3 1	2.6	1 5
70 WAT	0.2	2 1	3 3	1 2
104 WAT	0.3	2 9	2 9	1 7
Light (μmol/m²/sec)				
6 WAT	21	37	12	70
20 WAT	14	89	29	65
52 WAT	34	133	156	84
70 WAT	16	111	170	55
104 WAT	27	166	143	61

Tuber Production is Related to Rootcrown Density Following Biomass Recovery

In June, August, and November 1998, 1/4m² PVC frames were randomly placed in the untreated ponds, and three of the previously treated ponds that contained sprouting tubers to determine the number of rootcrowns that served as potential loci for tuber production. Results indicated that rootcrown density was lowest in the untreated ponds and dramatically increased from June through November in the treated ponds which were allowed to recover (Figure 4 6) Results from the mesocosm studies also showed a similar trend of decreasing rootcrown density the longer a thick vegetative canopy remains intact. Results suggest that intraspecific competition is limiting rootcrown density, even in shallow ponds Furthermore, based on light readings taken at the sediment surface in the untreated ponds sprouted tubers (as well as fragments) are not likely to establish under the dense canopy Without new loci for tuber production, the relative lack of new tubers formed in the untreated ponds is better explained.

Core samples collected in November 1998 confirmed the importance of rootcrown density in increasing tuber populations In the ponds that had been previously treated, tuber populations increased 36 to 117% of the background population measured in June and August 1998 (Figure 4 8). Based on these early numbers and the likelihood that tuber production will be maintained through May 1999, it is possible that tuber populations will exceed the original background population measured in May 1996

Fluridone Pore Water Concentrations Influence Tuber Establishment

As the studies progressed, hydrilla rapidly recovered following use of contact herbicides, and at 1 year posttreatment, tubers began establishing in the pond treated with liquid fluridone (AS). In contrast, it was noted that the pond treated with fluridone slow

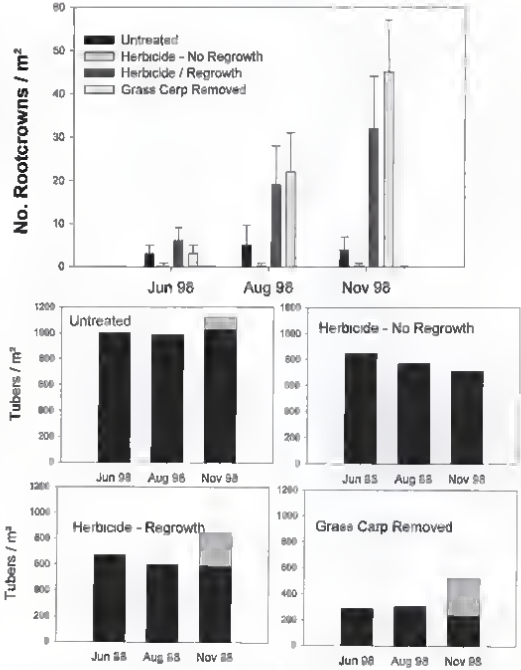

Figure 4.6 Hydrilla rootcrown and tuber density in research ponds at the Austin Cary Forest, FL. Ponds were left untreated, vigorously managed with herbicides (no hydrilla present) or they were allowed to recover from earlier herbicide treatments or grass carp herbivory. For rootcrown density, each bar represents the average of 10 (+ 1 SD) samples. For tuber density bars represent the average of 40 core samples. Light gray bars represent new tubers formed from September 1998 thru November 1998.

release pellets (SRP) did not contain any hydrilla at 52 WAT This was despite the fact that this pond had the overall highest density of tubers, and sprouting rates were not different from any of the other ponds Water samples collected at mid-depth in the water column did not result in detection of fluridone in the water column in either the AS or SRP pond through August 1997 Given the fact that fluridone could not be detected in the water column, the lack of hydrilla establishment in the SRP treated pond was not anticipated Results obtained from deploying sediment pore water equilibrators (peepers) in the SRP, AS and untreated ponds were evaluated and no fluridone was detected in the AS or untreated ponds In comparison, in the SRP treated ponds, fluridone was present in the sediment pore-water and at the sediment water interface at concentrations ranging from 3 to 6 µg/L (Figure 4 7) Previous laboratory studies have demonstrated that these concentrations are near the threshold fluridone concentrations to which mature hydrilla is sensitive (Netherland and Getsinger 1996, MacDonald 1993) These results likely explain the long term control of hydrilla sometimes achieved with fluridone SRP Furthermore, the ability to document that fluridone was in direct contact with *in situ* hydrilla tubers indicates that fluridone does not stimulate sprouting of quiescent tubers It is likely that following the granular treatments, sprouted tubers absorb fluridone in the pore water, and when exposed to light and oxygen upon emergence the rapid destruction of chlorophyll prevents establishment of these sprouting tubers.

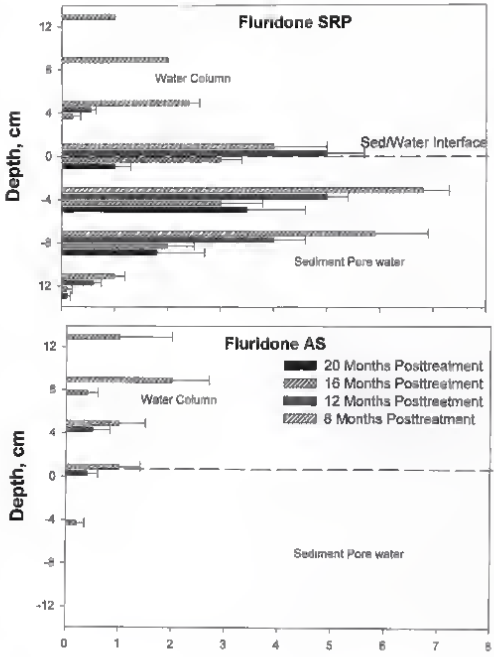

Figure 4 7 Sediment pore water concentrations of fluridone (SRP and AS) measured at 8 12, 16, and 20 months posttreatment in research ponds at the Austin Cary Forest, FL. Ponds were treated with either the granular (SRP) or liquid (AS) formulation of fluridone. Each bar represents the average of 3 replicates (+ 1 SD)

Summary and Conclusions

Intense management resulted in a slow decline in hydrilla tuber populations over a 30 month period, yet there was no evidence that sprouting rates were stimulated by any of the treatments compared to untreated controls. Tuber viability exceeded 90% throughout the study, suggesting that tuber survival was not influenced by age. Sprouting was not influenced by depth distribution in the sediment, tuber size, or sediment type. In contrast to tubers, sprouting of axillary turions was stimulated by canopy removal, with peaks noted the spring following herbicide treatment. Although sprouting tubers generally accounted for less than 2% of the population at any given sampling period, they resulted in rapid reinfestation of the ponds from May thru October. Results suggest that granular fluridone treatments prevented sprouting tubers from establishing (for up to 28 mo) due to the presence of low concentrations of fluridone in the sediment pore water and there was no evidence that fluridone treatment stimulated sprouting of hydrilla tubers. Rootcrown density was related to low rates of tuber production in untreated control ponds and to significant increases in tuber formation in newly recovering ponds.

Overall these results contradict several earlier hypotheses concerning the behavior of *in situ* tubers following intense management. Tuber sprouting was essentially the same under an untreated vegetative canopy as it was if all vegetation is slowly (fluridone) or rapidly removed (contact herbicides). It should be noted that due to the high density and relatively random distribution of tubers in these ponds, their ability to reinfest these small water bodies may not be indicative of results that occur in larger lakes. In larger water bodies, densities may be much lower and distribution is likely non random and clumped. Based on these studies, following hydrilla management there is likely a small

but generally continuous population of tubers that are sprouting, and their ultimate establishment is likely controlled by factors such as water quality (light penetration) and herbivory. The significant differences in sprouting behavior between tubers exposed to a drawdown and those that remain submerged (even under intense management) further support the contention of Sculthorpe (1967) that hydrilla evolved in a monsoon climate, and is well-adapted to wet and dry cycles. In conclusion, other than a drawdown, there is little that can be done with current technologies to stimulate sprouting of *in situ* hydrilla tubers.

CHAPTER 5
THE PARADOX OF TUBER SPROUTING LABORATORY EVALUATIONS TO DETERMINE FACTORS THAT STIMULATE AND INHIBIT *IN SITU* TUBER SPROUTING OF DIOECIOUS HYDRILLA

Introduction

Several investigators have conducted studies to determine factors that influence hydrilla tuber sprouting (Steward 1969, Haller et al 1976, Miller et al 1976, Bastouny et al 1978b, Sastrotomo 1980, Harlan et al 1985, Carter et al 1987, Kojima and Izawa 1989, Spencer and Ksander 1997) and essentially all of this research has relied on removing the tubers from the sediment and exposing them to conditions (light, oxygen, reduced CO_2) very different than exist in the hydrosoil. Consequently these studies have provided information on the viability of tubers, but have provided little or no useful information on factors that actually stimulate or inhibit *in situ* sprouting. It seems paradoxical that hydrilla tubers can remain quiescent in the sediment for years, yet removing them from the sediment generally results in sprouting rates that exceed 90% within 2 weeks of removal

Results of many studies suggest that sprouting of dioecious tubers can occur over a broad range of temperatures (15-35 C) with an optimum near 25 C (Haller et al. 1976, Steward and Van 1984, Harlan et al. 1985, and Kojima and Izawa 1989) Although suitable temperatures are required for sprouting based on results of *in situ* sampling in South Florida reported by Sutton and Portier (1985) and *in situ* tuber sprouting reported

in Chapter 4 of this study, changing temperatures are not likely a major stimulus for tuber sprouting Nonetheless, sprouting of many aquatic plant propagules is correlated with increasing spring temperatures (Madsen and Adams 1987, Spencer and Ksander 1992, Titus and Hoover 1991, Frankland et al 1987, Flint and Madsen 1995). Although it is reported that monoecious hydrilla tubers sprout as spring temperatures reach 11 to 15 C, there is no data on the percentage of the total population of tubers that actually sprout during this time In contrast to hydrilla, the *in situ* sprouting of many aquatic plant propagules is highly correlated with increasing sediment temperature in the spring (Madsen and Adams 1988, Flint and Madsen 1995, Spencer and Ksander 1992)

The role of light in the sprouting of *in situ* tubers is thought to be negligible due to limited light penetration (1-2 mm) in the hydrosoil (Benvenuti 1995) Nonetheless, light at levels as low as 12 μmol/m²/sec has been reported to stimulate sprouting rates of tubers removed from the sediment (Miller et al. 1976) The impact of light quality on tuber sprouting is equivocal as Miller et al (1976) reported light quality had no effect on sprouting rates, while Sastruotomo (1980) reported that both far-red and red light stimulated sprouting, and green and blue light inhibited sprouting The role of light in stimulating shoot or hypocotyl elongation is well-documented, and therefore observations that light stimulates rates of turion and tuber sprouting are not surprising A role for phytochrome mediated sprouting of turions has been suggested for both *Spirodela polyrrhiza* and *Potamogeton crispus* (Frankland et al 1987, Sastruotomo 1981) The lack of persistence of axillary turions of hydrilla compared to tubers may be related to the availability of light, and further research into the role of light on stimulating sprouting of axillary turions deserves research attention

The influence of exogenous hormone applications to inhibit or stimulate tuber sprouting has also been investigated on tubers removed from *in situ* conditions. Despite this limitation, compounds that reportedly stimulate the rates of sprouting such as gibberellic acid (GA) and ethylene (applied as Ethephon) have been identified (Steward 1969, Bastouny et al 1978a, Sastruotomo 1980); however, there are an reports that identify growth regulators that inhibit hydrilla tuber sprouting. Gaps identified in the hormone research conducted to date include the following 1) while exogenous hormone applications may stimulate sprouting rates, tubers generally sprout readily (up to 95%) within 2 weeks of removal from the sediment, regardless of hormone application 2) studies to date have ant tested the effects of known inhibitors of sprouting such as abscisic acid (ABA) or inhibitors of ethylene synthesis or action, and 3) hormone testing has not been conducted under the anoxic conditions which characterize the environment in which tubers are located (Jackson and Pearce 1991, Crawford 1992) Moreover, there is no information to indicate how long a tuber can be removed from the sediment before it is stimulated to sprout. Generation of this information would allow for improved study of tubers once they have been disturbed and removed from the sediment.

Although limited information exists for hydrilla, there are several reports to suggest that ABA, ethylene, and GA play significant roles in the sprouting of aquatic propagules (Weber and Nooden 1976, Winston and Gorham 1979, Anderson and Fellows 1993) ABA has also been shown to inhibit sprouting of *Cyperus rotundus* tubers (Teo and Nishimoto 1973), which display prolonged dormancy similar to hydrilla. To date there are no reports on the role that ABA and ethylene play in the sprouting response of hydrilla tubers. Ethanol accumulation due to anaerobic respiration has been reported

to stimulate or inhibit aquatic seed germination, as well as shoot production from submerged rhizomes (Smits et al. 1995 Braendle and Crawford 1987) Accumulation of ethanol and acetaldehyde as byproducts of anaerobic respiration have also been reported to both stimulate and inhibit sprouting of several aquatic species (Crawford et al 1987)

Laboratory studies have suggested that following removal of tubers from sediment, anoxic conditions (nitrogen environment) did not inhibit sprouting, whereas, exposure to elevated CO_2 concentrations (100% CO_2 gas) completely inhibited sprouting (Miller et al 1976) Following these initial characterizations, there had been little if any follow-up research conducted until recently Spencer and Ksander (1997) suggested that anoxic conditions actually stimulated the rate of sprouting of hydrilla and *Potamogeton* spp tubers, but did not impact the proportion of propagules sprouting Anoxia has been shown to have both stimulatory and inhibitory effects on sprouting of aquatic seeds and propagules of various plant species (Come et al. 1991) Due to the rapid response of hydrilla tubers to drawdown conditions it is strongly suspected that changes in the gaseous constituents of sediments influence sprouting Increased ethylene generation following a drawdown and subsequent reflooding is a well characterized phenomenon, and may play a key role in stimulating sprouting (Come et al 1991)

Ponnamperuma (1972) reports that the partial pressure of CO_2 in submerged sediments ranges between 0 05 and 0.2 atm (compared to 0 003 atm in the air), depending on soil properties and temperatures While anaerobic metabolism is predominant in sediments, the diffusion of oxygenated water into the sediment and flux of microbial generated CO_2 out of the sediments is slowed by approximately 10,000 times. Nonetheless, the transport of oxygen from submersed plant roots to sediments

(Sand Jensen et al. 1982, Sorrel and Dromgoole 1987, Jespersen et al 1998) that also contain high CO_2 concentrations, further complicates interpretation of factors that may influence *in situ* sprouting. Conditions created during a drawdown are also confounded by the fact that increased introduction of oxygen into newly created pore space is also accompanied by a significant reduction in CO_2 content.

In order to improve laboratory capabilities for studying factors that stimulate or inhibit tuber sprouting, a series of studies were conducted to both improve the methods currently used to handle tubers removed from the sediment, as well to better isolate the factors that may control tuber sprouting. It was important to determine if enhanced tuber sprouting is simply related to the initial disturbance (i.e. removal from the sediment) or the length of time the tuber remains removed from the sediment (anoxia) and exposed to a different set of environmental conditions (aerobic). The influence of light, hormone applications, ethanol, and gaseous constituents were tested under both anoxic and aerobic conditions in the laboratory in order to better understand and begin to isolate factors that may stimulate or inhibit tuber sprouting.

Materials and Methods

The Influence of Light on *in situ* Sprouting of Hydrilla Tubers

Plexiglass chambers (46 cm wide by 30 cm deep by 1.2 cm thick) described in Chapter 2 were filled with sediment collected from the Austin Cary research ponds. These sandy sediments were characterized by a dull gray color and low organic content (<4%). Twenty hydrilla apical shoots were planted in each of 12 chambers in October 1996 and September 1997. Chambers were placed in a 900-L concrete vault containing a

30 cm layer of hydrosoil. Chambers were pushed into the hydrosoil (30 cm deep) in order to prevent newly forming tubers from being exposed to light. Hydrilla grew rapidly and was allowed to form tubers during the fall, winter, and spring. In late May of 1997 and 1998, chambers were removed from the vaults and visible tubers growing on either side of the plexiglass were marked with a grease pen. Six chambers were removed from the sediment and placed in a 900 L vault that contained water, thus allowing these *in situ* tubers to be exposed to ambient light conditions. The remaining six chambers were placed back in the sediment to prevent any light from contacting the tubers.

For studies conducted in 1998, four redox probes were placed in three of the chambers from each treatment (light exposed and dark exposed tubers) with the platinum tips of two of the probes placed within 1 mm of a tuber, and the remaining two probes placed at least 5 cm from the nearest tuber. Depth of the probes ranged from 8 to 12 cm. Redox readings were taken at 4, 30, 70, and 120 d.

Chambers were visually evaluated at 30, 70, and 120 d for tuber sprouting. Tubers sprouting at each date were quantified and marked with a grease pen.

Tuber sprouting data for the 2 study years were pooled for further analysis. Pooled data of each sample period were compared by ANOVA and data at each sample date were compared using a t-test$_{0.05}$ in order to detect differences between treatments.

Tuber Removal/Replacement Studies

A series of studies was conducted to determine if simply removing the tuber from the sediment (disturbance) stimulated sprouting, or if the length of time the tuber remained exposed to different environmental conditions impacted sprouting rates. Potting soil was placed in a series of 1 L plastic pots and then flooded in 900 L concrete

vaults for 2 weeks to create reduced conditions in the hydrosoil (-217 to -309 mv). Large plastic flats that had supported hydrilla growth for several years in 900 L concrete vaults were used as a source of *in situ* tubers. Tubers were removed from these flats and placed 10 cm deep in the 1-L sediment-filled pots following their removal at 0.08, 0.5, 1, 5, and 30 minutes, and at 1, 2, 3, 6, 12, 24, 36, 48, and 72 hr intervals. Tubers that were removed were placed in distilled water in petri dishes allowing exposure to light, or in amber bottles to prevent exposure to light. At designated intervals, tubers were placed back into sediment-filled 1 liter pots at a depth of 10 cm. Pots were harvested at 30 and 90 DAT, with each treatment replicated 6 times.

Studies were conducted in February 1996 and 1997, April 1996 and 1997, June 1996 and 1997, August 1996 and 1997, October of 1996 and 1997. ANOVA indicated that no differences were detected for data collected between study years, and therefore data for each month were pooled for analysis. In contrast, significant differences in sprouting potential were noted between months and therefore data for each month are presented separately. Percent sprouting data were subjected to ANOVA and if differences were detected, data were further subjected to regression analysis. A t-test$_{0.05}$ was used detect differences in sprouting rates between individual treatments.

Tuber Sprouting in Response to Anoxic, Hypoxic, and High CO_2 Environments

All studies were conducted in a small growth room located at the Center for Aquatic Plants (CAP). Medical grade specialty gases were locally ordered (BITEC Industries) and the various combinations used are reported in Table 5.1. The CO_2 treatments were incorporated to test the potential of various concentrations of CO_2 to

inhibit sprouting, while CO_2 + low partial pressures of oxygen were tested to determine if low concentrations of oxygen could overcome any potential CO_2 inhibition. In general, hydrosoils have partial pressures of CO_2 ranging from 0 1 to 0 3 Atm, while molecular oxygen is absent (Ponnamperuma 1972) Nonetheless, in vegetated areas it is well documented that macrophyte roots release oxygen to the hydrosoil Therefore, it is feasible that a tuber may exist in an environment of both high CO_2 and low oxygen

The experimental apparatus used for these studies consisted of a series of 150 mL Erlenmeyer flasks that were initially filled with 100 mL of distilled water and then tightly sealed with a rubber stopper containing an inlet and exhaust valve. A series of six flasks were connected (outlet to inlet) with Tygon tubing and vigorously sparged with a selected gas for a period of 45 minutes Plastic flats (Chapter 2) containing *in situ* tubers were brought into the growth room A small fluorescent light covered with a green filter was placed in the corner of the room and served as the only source of light during the tuber sorting and evaluation periods *In situ* tubers removed from the flats were rinsed in distilled water and immediately placed (within 30 seconds) in the 150 mL flasks Ten tubers were placed in each flask, and flasks were continuously sparged for another 30 minutes At this time tubing was either connected (inlet to outlet) to form a static exposure, or gas flow rates were greatly reduced to 0 7 L/hr in order to create a flow-thru system to remove buildup of potentially volatile toxic products (ethanol, acetaldehyde) that can result from anoxic exposure (Crawford et al 1987) For the flow-thru studies, a seventh flask that did not contain any tubers was attached at the end of the series. This flask was completely filled with distilled water, and it was added to exclude potential influx of air to the gas mixture

Table 5 1 Gas mixtures used for laboratory evaluations of dioecious hydrilla tuber sprouting

Compressed Air - Nitrogen (78%) + Oxygen (21%) + carbon dioxide (0 03%)
Nitrogen (100%)
Nitrogen (99%) + Carbon dioxide (1%)
Nitrogen (93%) + Carbon dioxide (7%)
Nitrogen (86%) + Carbon dioxide (14%)
Nitrogen (89%) + Carbon dioxide (7%) + Oxygen (4%)
Nitrogen (83%) + Carbon dioxide (7%) + Oxygen (10%)

Sprouting was evaluated at 14, 28, and 56 DAT Evaluations were conducted under a green-filtered fluorescent light placed in the corner of the room, otherwise, the environment remained dark throughout the study Studies were initiated in April of 1996 and 1997, June of 1996 and 1997, and in December of 1996 and 1997

Initial studies suggested a toxic response to treatments utilizing CO_2 It was noted that sparging distilled water rapidly drove the pH below 4 5 (range of 3.2 to 4 5), and it was hypothesized that the resulting acidic conditions were responsible for tuber toxicity Use of CO_2 as a competitive inhibitor of ethylene has often been discouraged due to confounding effects due to reduced cellular pH (Reid 1995) To overcome a suspected pH-induced toxicity, $NaHCO_3$ buffer (4 mM) was added to the flasks to resist pH changes following addition of CO_2 gas The pH in the buffered flasks sparged with CO_2 equilibrated to 5 3 to 6.2 following the vigorous CO_2 sparging process Several pilot studies suggested that tubers in untreated control flasks to which buffer was added

sprouted at the same rate as tubers in flasks which were not buffered. Therefore, buffer was added to all treatment flasks.

Data were pooled for the studies and were subjected to ANOVA to test for treatment effects (WAT, gas exposure) and interactions. The effect of gas mixture was separated using an $LSD_{0.05}$ at each evaluation period.

Tuber Sprouting in Response to Various Hormones and Ethanol

Studies were conducted in a growth room located at the Center for Aquatic Plants and in an environmental chamber located in Vicksburg, MS (USAE WES). Hormone applications were conducted under both anoxic conditions (nitrogen environment as described above) and under aerobic conditions. Studies were initiated in April and July of 1996 and 1997. Hormone treatment concentrations are listed in Table 5.2.

Treatments conducted under anoxic conditions used an experimental apparatus and protocol as described above. For the aerobic treatments, petri dishes were filled with 50 mL of distilled water and stock concentrations of the selected hormone were applied. *In situ* tubers were removed from plastic flats, rinsed with distilled water and immediately placed in the experimental containers. Ten tubers were placed in each flask.

Each treatment was replicated 6 times and evaluations to determine percent sprouting were conducted at 1, 2, 4, and 8 WAT. Tests conducted with single hormones were subjected to ANOVA and if significant differences were detected, percent sprouting rates were separated by an $LSD_{0.05}$. Analyses indicated no differences existed between studies conducted in 1996 and 1997 and therefore data were pooled for analysis. For studies in which ABA and GA were combined, treatment and interaction effects on tuber sprouting were subjected to ANOVA.

Table 5 2 Hormone treatments conducted in the laboratory to determine effects of sprouting of hydrilla tubers in both anoxic and aerobic conditions.

Treatment	Concentration	Treatment	Concentration	Environment
	µM		µM	
Untreated				Aerobic
Untreated				Anoxic
ABA	0 05, 0 1, 0 5, 1 0, 10			Aerobic
ABA	0 05, 0 1, 0 5, 1 0, 10			Anoxic
ABA	0 1, 1 0, 10 0	**GA**	1, 15, 150	Aerobic
ABA	0 1, 1 0, 10 0	**GA**	1, 15, 150	Anoxic
AVG	0 1, 0 25, 0 5, 1 0			Aerobic
	0 1, 0 25, 0 5, 1 0			Anoxic
Silver Thiosulfate	100, 500, 1000, 5000, 10 000			Aerobic
	100, 500, 1000, 5000 10,000			Anoxic
Ethanol	1000, 5000, 10,000, 25,000 50,000			Aerobic
	1000, 5000, 10,000, 25,000, 50,000			Anoxic

Tuber Removal Replacement Studies with ABA

Previous studies showed that the length of time a tuber was removed from the sediment impacted its ability to sprout (Figure 5 1), and once the sprouting response was initiated, placing the tuber back in the sediment did not inhibit sprouting This observation was further tested using the hormone ABA Studies were conducted in April, June, and August of 1998 *In situ* tubers were removed from plastic flats as described above, and placed in petri dishes containing distilled water for 0 15, 0 5, 1, 5, 30, and 60

minutes, and 2, 6, 12, 24, 48, and 72 hr. Following the designated time in distilled water, ten tubers were placed in petri dishes containing 50 ml of distilled water treated with a 1.0 μM concentration of ABA. Treatments were replicated 6 times and conducted under aerobic conditions. Percent sprouting in ABA treated petri dishes was evaluated at 30 and 60 DAT.

ANOVA indicated differences existed between the studies and therefore data from each study were analyzed separately. Data were subjected to ANOVA and if differences in sprouting were noted, data were further subjected to regression analysis.

Gibberellin Synthesis Inhibitor Effects on Tuber Sprouting

Based on results of laboratory studies that suggested a role for GA and ABA in tuber sprouting, the gibberellin synthesis inhibitor flurprimidol ((a-(1-methylethyl)-a-(4-(trifluoromethoxy)phenyl)-5-pyrimidine-methanol) was used to treat 20 plastic flats that were planted with hydrilla in November 1997. Flurprimidol was added to the 900 L mesocosm vaults at a rate of 75 μg/L in December 1997, and these treatments were compared to a set of 20 untreated controls that were also established in November 1997. On May 3, 1998, all flats were treated with endothall at 5.0 mg/L to remove the vegetative canopy. Previous mesocosm studies (see Chapter 3) had demonstrated that contact herbicides had a stimulatory impact on tuber sprouting in the shallow flats.

Five containers from each treatment were collected on May 3, June 20, August 18, and September 21, 1998. Total tuber number (% sprouting, rotting, and quiescent) and shoot biomass were quantified. In this study, the tuber sprouting response following removal from the sediment was the critical parameter to be monitored. Data at each harvest date for flurprimidol-treated and untreated flats were compared by a t test$_{0.05}$

Influence of Anoxia on Quiescent Tubers

A small proportion of tubers (<4%) often remained quiescent following viability studies conducted in the laboratory (Chapter 2 and 4). These tubers remained quiescent for months even though conditions for sprouting were considered favorable. Following viability tests of tubers collected from Rodman Reservoir in March 1996, over 100 quiescent tubers were saved for future studies. These quiescent tubers remained in petri dishes filled with distilled water for 120 days. In July 1996, a subset of these tubers were placed in petri dishes that had been filled with water saturated potting soil. Six tubers were placed in each sediment-filled petri dish and four petri dishes were collected at 14 and 35 DAT. Untreated control tubers remained in the petri dishes filled with distilled water. These studies were repeated with tubers collected from the Austin Cary ponds in April 1997 and February 1998. Tubers collected from Austin Cary also remained quiescent for over 120 days prior to initiation of the studies.

All experiments were conducted under dark conditions. Redox probes placed in the petri dishes prior to initiation of the studies indicated that these sediments were highly reduced (-167 to -286 mV). Sprouting data at each sample date were subjected to a t-test$_{0.05}$ to determine if differences existed between tubers that remained in distilled water and those placed back in the reduced sediments.

Results and Discussion

Exposure to Light Increased *in situ* Tuber Sprouting

The number of visible tubers that formed during the winter months in the plexiglass chambers ranged from 17 to 26 (mean = 20). A significant interaction

($p<0.05$) on sprouting was noted between DAT and exposure to light or dark conditions. Sprouting percentages of tubers in plexiglass chambers that were exposed to light were significantly increased compared to tubers that remained under dark conditions (Table 5.3). However, over 68% of the tubers exposed to light conditions remained quiescent.

Table 5.3. Comparison of *in situ* tuber sprouting in sediment-filled plexiglass chambers exposed to light and dark conditions.

DAT	Treatment	
	Light	Dark
30	4	6
70	21*	8
120	32*	11

[1]Values followed by an asterisk indicate differences between treatments at a give sampling date according to a t test$_{0.05}$.

past 120 d. Redox data were variable between chambers, but sediments remained reduced throughout both light and dark treatments. While these studies are the first to report on the impact of light on the sprouting of *in situ* quiescent tubers, there may have been confounding factors that stimulated sprouting.

The majority of the tubers in the light exposed chambers began to turn green (chlorophyll was produced) by 30 DAT and were presumably photosynthetic. Although no differences in redox were detected between probes placed within 2 mm of tubers and those that were at least 5 cm from the nearest tuber, production of oxygen by photosynthetic tubers cannot be discounted. The release of photosynthetically derived oxygen into the microenvironment surrounding the tuber could potentially impact redox

chemistry as well as carbon dioxide concentrations. Therefore while exposure to light increased tuber sprouting, the factors responsible for this increase remain unknown

Time of Removal from the Sediment Influenced Tuber Sprouting and Mortality

Sprouting rates were greatly affected by the length of time tubers were removed from *in situ* conditions in the sediment. Exposing tubers to light or dark conditions did not influence sprouting, and these data were combined for regression analysis. While studies conducted in February, April, June, and October generally resulted in similar trends in the sprouting response, results of the August studies were dramatically different. Overall results suggest that the sprouting response was stimulated within 30 minutes after removing tubers from the *in situ* environment (Figure 5.1). Differences in overall sprouting rates at different times of the year were likely influenced by ambient environmental conditions (i.e. temperature), however, it should be noted that much higher overall sprouting rates were observed in August and October (70-95%), suggesting these tubers were predisposed to sprout. A similar trend of maximal sprouting in October/November for tubers collected in the field was noted in Chapter 4. Madsen and Owens (1998) report maximal sprouting of hydrilla tubers in Central Texas occurs in August.

Sprouting rates for tubers removed from the sediment for 5 seconds through 5 minutes generally remained below 15%, and no differences (according to a $t\text{-test}_{0.05}$) were noted between any of these treatments. The fact that tuber sprouting was observed within 30 days of initiating these studies suggests that removing the tubers from the sediment stimulated a rapid response that is similar to laboratory observations in petri dishes.

While maximal sprouting was generally observed for those tubers removed from the sediment for 24 to 48 hr, this response dropped significantly by 72 hr (Figure 5.1).

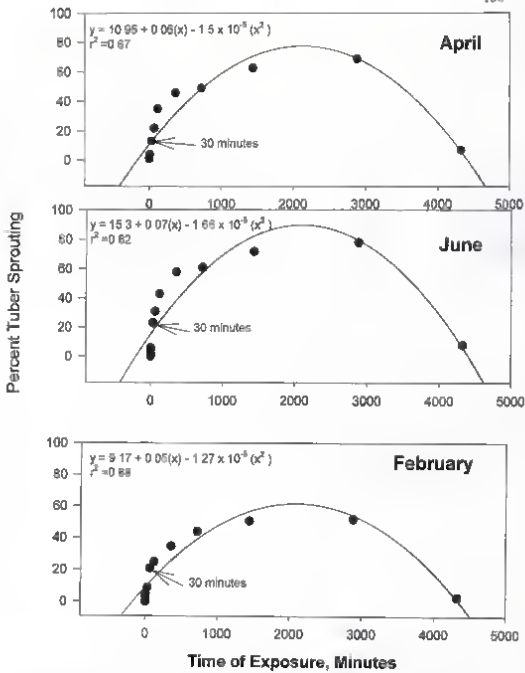
y = 10 95 + 0 06(x) - 1 5 x 10⁻⁵ (x²)
r² =0 67
April
30 minutes
y = 15 3 + 0 07(x) - 1 66 x 10⁻⁵ (x²)
r² =0 82
June
30 minutes
y = 9 17 + 0 06(x) - 1 27 x 10⁻⁵ (x²)
r² =0 68
February
30 minutes
Percent Tuber Sprouting
Time of Exposure, Minutes

Figure 5 1 Regression equations used to predict the time required to stimulate tuber sprouting following removal from the sediment Studies were initiated in April, June, and February Tubers were removed from the sediment for a designated time and then placed back in reduced sediments Decreased sprouting rates at 72 hr were attributed to increased mortality rates Sprouting was determined at 30 DAT and each point represents the average of 6 replicates.

Low sprouting rates for this treatment can be explained by the fact that mortality rates ranged from 79 to 94% for these tubers. In general, tubers removed from the sediment for 72 hr and then placed back in the sediment were completely rotten by the 30 day evaluation. While increased mortality explains the reduction in sprouting, factors contributing to the increased mortality are not understood. It should be noted that increased mortality was also observed for tubers exposed for 24 hr (13-21%) and 48 hr (16-27%). In contrast, tubers removed from the sediment for less than 12 hr suffered mortality rates of less than 5%.

The sprouting response observed for studies conducted in late August differed significantly from trends observed in other studies (Figure 5.2). Although differences were noted between studies conducted in 1996 and 1997, increased sprouting following any disturbance was consistent. Studies conducted in 1996 resulted in lower sprouting rates (40-55%) for tubers removed from the sediment for less than 5 minutes, however, in 1997 any level of disturbance significantly increased sprouting rates above 70 percent.

Results indicate that hydrilla tubers may be especially pre-disposed toward sprouting in August (possibly through October) given a slight external stimulus. In the studies conducted in August, it appears that simply disturbing an *in situ* tuber, greatly increased sprouting rates, but further confirmation is needed.

With the exception of studies initiated in August, results suggest that tubers can be removed from the sediment for short periods of time without stimulating sprouting. Sprouting rates did not increase between 30 and 90 days, indicating that following an initial rapid stimulation of sprouting, a long-term delay in sprouting response was unlikely for the various treatments. The results of this study explain the existence of

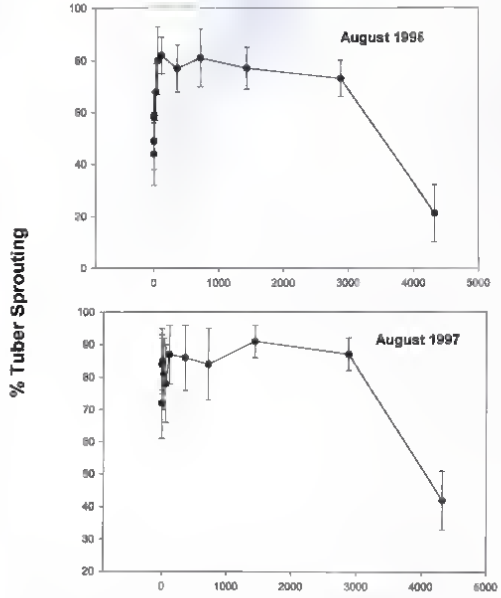

Figure 5 2. Time required to stimulate tuber sprouting following removal from the sediment. Studies were initiated in August. Tubers were removed from the sediment for a designated time and then placed back in reduced sediments. Decreased sprouting rates at 72 hr were attributed to increased mortality rates. Sprouting was determined at 30 DAT and each point represents the average of 6 replicates.

different sprouting rates from *in situ* (<5%) to artificial conditions (>90%) and brings into question other studies which have employed the use of tubers which have been removed from the sediment for an unknown length of time It is likely that tubers harvested to the field and held under aerobic conditions for any length of time prior to testing likely confounded study results These studies indicated that further laboratory testing with mixed gases and hormones on "quiescent tubers" is needed, provided tubers are immediately placed under treatment conditions While the factors that stimulated sprouting were not determined (experiments did suggest that light was not a factor in sprouting), the sprouting response was initiated rapidly, occurring within 30 minutes to 1 hour A linear relationship between time removed from the sediment and increased sprouting was noted through 48 hr Once initiated to sprout, placing these tubers back into reduced sediments (which generally inhibit the sprouting response) did not result in inhibition of sprouting

Differences in sprouting between tubers collected in August versus other times of the year are intriguing and lead to development of several hypotheses for testing Nonetheless, the overall objective of this work was to determine if methods could be developed to allow for the use of "quiescent" tubers for laboratory evaluations With the exception of tubers collected in August, these studies suggest that immediate placement of *in situ* tubers in laboratory treatments may allow evaluation of tubers that are not predisposed to sprout.

Carbon Dioxide Inhibited Sprouting of Hydrilla Tubers

A significant two-way interaction ($P < 0.05$) for tuber sprouting existed between the gas mixtures and DAT ANOVA indicated that no differences in tuber sprouting

existed between the static and flow through systems, however, data are presented for static exposures only

Treatments incorporating CO_2 reduced tuber sprouting compared to untreated controls (Table 5 4) In contrast, tubers in a nitrogen environment (anoxic) sprouted at a similar rate to untreated controls Although overall sprouting rates differed between study years the effect of treatments on sprouting trends remained similar between studies.

Addition of low partial pressures of oxygen in the presence of CO_2 did not stimulate tuber sprouting compared to CO_2 alone (Table 5 4) It was hypothesized that addition of oxygen would allow conversion of aminocyclopropane-1-carboxylic acid (ACC) to ethylene, so oxygen was added to the high CO_2 environment in order to determine if anoxia and high CO_2 were combining to prevent action of ethylene

Sparging flasks with CO_2 generally dropped the pH below 4.5 and reduced sprouting observed in unbuffered systems was attributed to a pH-induced toxicity (data not shown) Buffering the water both increased tuber sprouting and reduced tuber mortality at 56 DAT, however, mortality rates still remained quite high (24-53%) in these buffered flasks It was also noted that in some cases high rates of tuber mortality (up to 25 %) occurred under the nitrogen environment (pH 6 5) by 56 DAT In general higher rates of mortality were observed in the static versus flow-through treatments, suggesting that a toxic buildup of ethanol or acetaldehyde (due to anaerobic respiration) may have also played a role in tuber mortality The addition of ten tubers to a small volume of water (150 mL) may have exacerbated the toxic response.

Complicating interpretation of the data was the fact that living and dead tubers in most cases were not easily distinguishable The majority of dead tubers remained

Table 5 4 Hydrilla tuber sprouting in the laboratory following exposure to various gas environments

Treatment	Days After Treatment		
	14	28	56
		April	
Untreated (Aerobic)	82	91	91
Nitrogen	83	89	93
1% CO_2	37	46	46
7% CO_2	28	35	35
14% CO_2	8	8	8
7% CO_2 + 4% 02	24	29	34
7% CO_2 +10% 02	24	34	34
LSD[1]	7	11	8
		June	
Untreated (Aerobic)	87	89	89
Nitrogen	69	78	85
1% CO_2	37	46	46
7% CO_2	28	32	32
14% CO_2	11	13	13
7% CO_2 + 4% 02	21	29	38
7% CO_2 +10% 02	27	34	39
LSD	12	7	8
		December	
Untreated (Aerobic)	27	48	63
Nitrogen	29	47	56
1% CO_2	11	19	24
7% CO_2	12	15	20
14% CO_2	3	3	3
7% CO_2 + 4% 02	14	17	19
7% CO_2 +10% 02	21	25	25
LSD	6	7	11

[1]Treatments within each month and sample date were compared by an $LSD_{0.05}$ to determine if differences existed between treatments.

structurally intact in the flasks, yet they had become soft (healthy tubers are quite turgid) and the apical shoots became detached with just a small provocation

While CO_2 at concentrations as low as 1 to 14% inhibited tuber sprouting, it is recommended that these data be viewed with some caution In most cases the gas treatments became toxic to the tubers and it was not determined when this mortality occurred. It is worth noting that harvests conducted in 1997 at 2 WAT suggested that >90% of the tubers were still viable (i e. the toxic effect was not immediate and could be overcome)

Previously, Miller (1975) reported that tuber sprouting was inhibited under a CO_2 environment. In reviewing the methods, these studies were conducted in a 100% CO_2 environment, and the distilled water source was not buffered. The long-term results observed in these studies were possibly due to the fact that the pH was likely below 4 0, resulting in these treatments acting as a preservative

Addition of ABA Inhibited Tuber Sprouting

Addition of exogenous hormones resulted in identification of ABA as a strong inhibitor of tuber sprouting under both anoxic and aerobic conditions (Table 5 5) In contrast, while GA slightly stimulated sprouting rates silver thiosulfate (STS), aminoethoxyvinylglycine (AVG), and ethanol only had impacts on tuber sprouting at the highest range of concentrations tested under both aerobic and anoxic conditions (Tables 5 6 and 5 7) In the case of ethanol treatments of 2 5 to 5 0% (25,000 to 50,000 µM), the inhibition of sprouting was more likely due to a narcotic effect of the ethanol It is interesting to note that tuber mortality increased in the presence of higher concentrations of ethanol, further suggesting a toxic role for this compound in the earlier gas studies

Table 5 5 Hydrilla tuber sprouting following addition of abscisic acid (ABA) under both aerobic and anoxic conditions

Treatment (μM)	Weeks After Treatment 1	2	4	8
		April 1996-97		
ABA (anoxia)				
0	56	87	91	95
0 05	26*	47*	76*	76*
0 1	9*	16*	37*	49*
0.5	0*	7*	8*	11*
1 0	0*	3*	4*	6*
5 0	0*	3*	3*	3*
10 0	0*	0*	0*	0*
ABA (aerobic)				
0	63	79	96	96
0 05	31*	44*	64*	77*
0 1	11*	19*	39*	52*
0.5	0*	11*	11*	11*
1 0	0*	5*	10*	10*
5 0	0*	0*	0*	0*
10 0	0*	0*	0*	0*
		June 1996-97		
ABA (anoxia)				
0	71	79	96	96
0 05	38*	48*	59*	64*
0 1	9*	16*	37*	49*
0 5	0*	7*	8*	11*
1 0	0*	3*	4*	6*
5 0	0*	3*	3*	3*
10 0	0*	0*	0*	0*
ABA (aerobic)				
0	68	82	96	96
0 05	21*	39*	67*	73*
0 1	11*	19*	39*	52*
0 5	0*	11*	11*	11*
1 0	0*	5*	10*	10*
5 0	0*	0*	0*	0*
10 0	0*	0*	0*	0*

*Treatments are different from untreated controls according to a Dunnet's test at the 0 05 level of significance (n=6)

Table 5 6 Hydrilla tuber sprouting following addition of gibberellic acid (GA_3) and ethanol under both aerobic and anoxic conditions

Treatment (μM)	Weeks After Treatment 1	2	4	8
GA_3 (anoxia)				
0	63	83	94	96
15	72*	83	90	95
50	79*	95*	97	97
100	69*	94*	96	96
GA_3 (aerobic)				
0	66	81	96	96
15	77*	88*	92	95
50	74*	93*	95	97
100	82*	96*	96	96
Ethanol (anoxia)				
0	63	83	94	96
1000	77*	81	96	96
5000	79*	88	93	95
10000	67	79	88	93
25000	22*	34*	38*	38*
50000	0*	2*	2*	2*
Ethanol (aerobic)				
0	66	81	96	96
1000	71	84	91	98
5000	65	74	91	91
10000	62	79	93	93
25000	38*	44*	46*	46*
50000	0*	0*	0*	0*

*Treatments are different from untreated controls according to a Dunnet's test at the 0 05 level of significance (n=6)

Table 5 7 Hydrilla tuber sprouting following addition of silver thiosulfate (STS) and aminoethoxyvinylglycine (AVG) under both aerobic and anoxic conditions

Treatment (μM)	Weeks After Treatment 1	2	4	8
STS (anoxia)				
0	63	83	94	96
100	57	76	87	98
500	55	87	98	98
1000	60	78	86	93
5000	22*	54*	73*	73*
10000	4*	8*	8*	8*
STS (aerobic)				
0	66	81	96	96
0 1	71	84*	94	96*
0 5	63	78*	91	94*
1 0	59	85*	90	93*
5 0	14*	37*	60*	60*
10 0	0*	6*	6*	6*
AVG (anoxia)				
0	63	83	94	96
0 1	64	75	91	91
0 25	71	73	92	94
0 5	58	72	89	89
1 0	66	81	93	93
AVG (aerobic)				
0	66	81	96	96
0 1	58	84	92	95
0 25	62	86	95	95
0 5	67	78	85*	89
1 0	54*	73	88	93

*Treatments are different from untreated controls according to a Dunnet's test at the 0 05 level of significance (n=6)

The relative lack of STS and AVG activity under aerobic conditions was somewhat surprising. Under anoxic conditions ethylene biosynthesis is prevented due to lack of molecular oxygen, and therefore the lack of STS and AVG activity would be expected. Nonetheless ethylene synthesis generally resumes when tissues that have been under anoxic conditions are re-exposed to air (Reid 1995). Although higher concentrations of STS inhibited tuber sprouting, subsequent viability tests at the end of these studies suggest that this reduction was more likely due to a toxic effect on the tubers. While STS is often recommended as an inhibitor of ethylene action due to its low phytotoxicity (Reid 1995), results of this study suggest that continuous exposure of tubers to higher concentrations of silver were toxic.

Based on results of studies with inhibitors of ethylene action, the inhibitory effects of a high CO_2 environment (1-14%) on tuber sprouting become more difficult to explain on a physiological basis. CO_2 has been used as an inhibitor of ethylene synthesis for years, however, other possible effects of CO_2 (e.g. inhibition of respiration and effects on cellular pH) often confound observations. The fact that STS and AVG had limited activity on tubers at physiological concentrations suggests that CO_2 effects observed in this laboratory studies may not be due to competitive inhibition of ethylene action.

There was a significant difference ($p < 0.05$) for tuber sprouting among treatments and a significant interaction ($p < 0.05$) between ABA, GA, and DAT. While ABA at concentrations of 0.1, 1.0 and 10 µM greatly inhibited sprouting, addition of GA at a concentration of 15 µM could often overcome this inhibition (Table 5.8). Addition of GA at a 150 µM concentration resulted in both stimulatory and inhibitory effects depending

Table 5 8 Percent sprouting of hydrilla tubers at varying concentrations of ABA and GA

Treatment, μM	Weeks After Treatment			
	1	2	4	8
Untreated	68	82	93	94
ABA				
0 1	11*	16*	26*	26*
1 0	2*	4*	4*	4*
10 0	0*	0*	2*	2*
GA				
1 0	63	79	90	96
15 0	84*	93*	96	96
150 0	86*	89	94	95
ABA + GA				
0 1 + 1 0	15*	20*	37*	37*
0 1 + 15 0	64	76	85	91
0 1 + 150 0	60	86	91	93
ABA + GA				
1 0 + 1 0	5*	8*	8*	8*
1 0 + 15 0	65	85	91	96
1 0 + 150.0	11*	15*	19*	19*
ABA + GA				
10 0 + 1 0	4*	9*	13*	18*
10 0 + 15 0	12*	16*	22*	25*
10 0 + 150 0	6*	6*	15*	19*

Treatments are different from untreated controls according to a Dunnet's test at the 0 05 level of significance (n=6)

of the rate of ABA applied Application of 10 µM ABA remained inhibitory regardless of the rate of GA added to counteract the effect

These results contradict those reported by Thakore (1996), who suggested that ABA stimulated and GA inhibited the sprouting of axillary turions of monoecious hydrilla. It should be noted that sprouting of untreated control turions was below 20%. Results of the current studies suggest that ABA is a strong inhibitor of sprouting for dioecious hydrilla tubers

MacDonald (1994) demonstrated that ratios of ABA to GA controlled hydrilla tuber formation, and that disruption of ABA synthesis with the herbicide fluridone could prevent tuber production Data reported in these studies suggest that ABA and GA play a key role in the sprouting of hydrilla tubers. The role of these hormones in germination of seeds and propagules is well documented in terrestrial plants (Hillhorst and Karssen 1992) and several studies have demonstrated a role for reduced ABA concentrations being related to sprouting of aquatic propagules (Weber and Nooden 1976, Winston and Gorham 1979, and Anderson and Fellows 1993) Nonetheless, these studies provide the first evidence of the potential role for ABA and GA in sprouting of hydrilla tubers

Time of Exposure to Aerobic Conditions Influenced ABA Impact on Sprouting

Following the removal of tubers from the sediment, the length of time that elapsed prior to placing them in a 1 µM ABA solution significantly impacted tuber sprouting (Figure 5.3). Tubers exposed to aerobic conditions for less than 1 hour generally resulted in similar sprouting rates, however, as time of exposure increased there was a linear increase in tuber sprouting. Although these results compare well with the removal/replacement studies described above, two substantial differences were noted

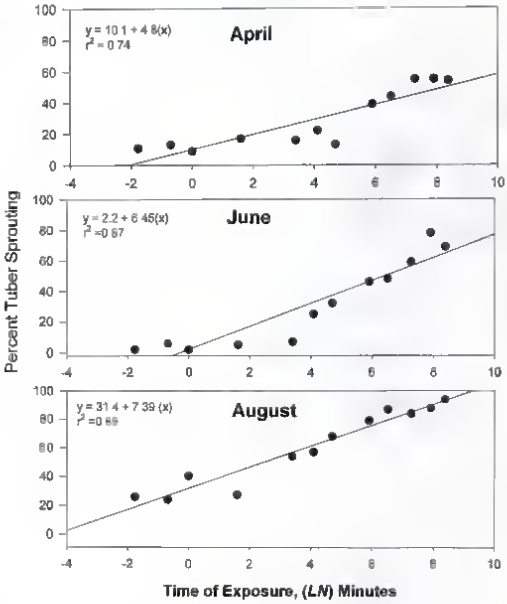

Figure 5 3 Regression equation used to predict the time required to stimulate tuber sprouting following removal from the sediment Studies were initiated in April June, and August. Tubers were removed from the sediment for a designated time and then placed in a 1 µM solution of ABA Sprouting was determined at 30 DAT and each point represents the average of 6 replicates

First, sprouting rates in the laboratory studies were generally higher for those tubers exposed for less than 1 hour. Furthermore, laboratory studies indicated that a 72 hr exposure resulted in increased sprouting rates, whereas placing tubers back in the sediment at 72 hours resulted in high mortality rates. Sprouting rates were generally highest during August, however, in contrast to the earlier removal/replacement studies using sediment, a linear response in tuber sprouting was noted for the laboratory studies using ABA.

These studies further suggest a role for ABA in the sprouting of hydrilla tubers, and research to characterize internal ABA concentrations is suggested. While there is sometimes a lack of correlation between ABA content of seeds and their physiological behavior (Walton 1980), recent research by Le Page-Degivry et al. (1997) suggest that dormancy breaking can be associated with changes in ABA synthesis or catabolism. Anderson and Fellows (1993) report a notable reduction in ABA content of *Potamogeton nodosus* tubers just prior to their sprouting. Measurement of changes in ABA content of tubers (of a known age) following their removal from the sediment may provide more precise information on the role of ABA in hydrilla tuber sprouting.

Gibberellin Synthesis Inhibitor Treatment Inhibits Tuber Sprouting

To further test the potential role of ABA and GA in an *in situ* environment, the gibberellin synthesis inhibitor flurprimidol was used to treat flats containing new established hydrilla. Tuber production in the flurprimidol-treated flats (36 ± 10) was reduced compared to production observed in untreated flats (56 ± 9). Flurprimidol treatment resulted in plants forming a thick vegetative mat near the sediment as opposed to the surface canopy observed in the untreated plants. Despite differences in

morphology, biomass production remained similar between both treatments (Figure 5 4) By August, the flurprimidol treatment effects diminished and plants began to elongate to form a surface canopy Visual observation suggested that root density was similar between flurprimidol treated and untreated plants

Harvests in May indicated that in both untreated and flurprimidol-treated flats, less than 4% of the tubers collected had sprouted Following endothall treatment to remove the vegetative canopy, untreated hydrilla (no flurprimidol applied) displayed a marked increase in tuber sprouting compared to flurprimidol-treated plants. These results suggest that the GA inhibitor flurprimidol inhibited the ability of tubers to sprout.

Removal of tubers from the sediment in May and June resulted in over 94% of untreated tubers sprouting within 10d, whereas, < 4% of the flurprimidol-treated tubers sprouted by 21 days (Figure 5 4) In fact, tubers from flurprimidol treated vaults had not sprouted by 90 days after removal from the sediment. Application of exogenous GA (20 μM) resulted in 38% sprouting of these quiescent propagules In contrast to the earlier harvests, the July harvest resulted in approximately 39% of the flurprimidol-treated tubers sprouting within 10-d and 65% by 30-d after removal from the sediment.

Sprouting rates of untreated tubers continued to exceed 90% within 7-d of removal from the sediment. Although there was very limited *in situ* sprouting observed during the August harvest, approximately 68% of flurprimidol treated tubers sprouted within 10 d and 85% by 30-d after removal from the sediment. While the flurprimidol effects on tuber sprouting diminished with time, it should also be noted that the growth regulating effects of the flurprimidol on the vegetative canopy had also begun to subside by August

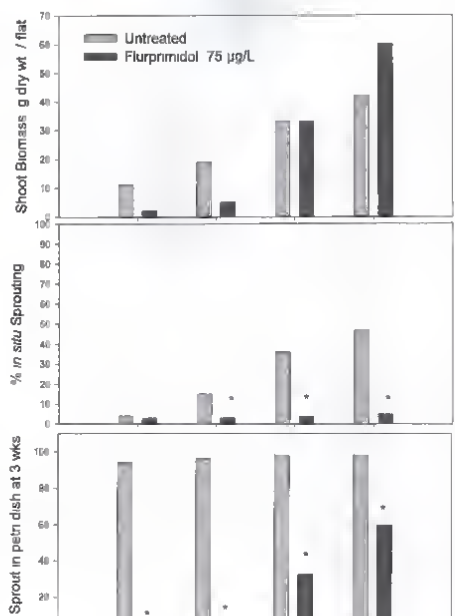

Figure 5 4 Mesocosm evaluation of the impact of flurprimidol treatment on shoot biomass, percent *in situ* tuber sprouting, and percent sprouting in petri dishes. Asterisks above the bars indicate differences between untreated and flurprimidol-treated flats according to a t-test$_{0.05}$ (n=5)

These results suggest that flurprimidol inhibited tuber sprouting through the spring and summer following a December application Flurprimidol has a half-life of >100 d in the hydrosoil, and therfore these results do not allow determination of whether the inhibition of tuber sprouting is due to altered GA/ABA ratios during the formation of the tuber, or if flurprimidol in the pore water was exerting an external influence on the detached tubers

A previous laboratory bioassay study indicated that application of flurprimidol (50 to 100 µg/L) significantly increased axillary turion production, however, these turions would not sprout unless supplied with exogenous GA (Netherland 1989) The role of ABA and GA in the formation of hydrilla tubers is well documented (MacDonald 1994), and results from this study suggest that these hormones also play a role in quiescence and sprouting of hydrilla tubers

Quiescent Tubers Readily Sprouted When Placed in Anoxic Conditions

Placing quiescent tubers (quiescent > 100 d) back in the sediment under reduced conditions significantly stimulated sprouting rates compared to tubers that remained in distilled water (Table 5.9) The majority of tubers in the distilled water remained quiescent for over 1 year (data not reported) Sprouting rates in the sediment increased greatly between 14 and 35 days, suggesting that the stimulus that initiated sprouting was somewhat delayed- It should be noted that tubers used for this study represented a very small fraction (<4%) of the total population While greater than 90% of tubers generally sprout within 2 weeks of removal from the sediment, a small population of tubers remain quiescent, yet viable Pilot studies with GA and ACC at several rates suggested this quiescence could not be overcome with addition of these compounds

Table 5 9 Sprouting response of tubers that had remained quiescent for > 100 d, when placed back in reduced sediments

Source of Tubers	% Sprouting at 14 DAT: Reduced Sediment	% Sprouting at 14 DAT: Distilled Water
Rodman Reservoir (6/96)	43*	0
Austin Cary Ponds (8/97)	37*	0
Austin Cary Ponds (5/98)	57*	1
	% Sprouting at 35 DAT	
Rodman Reservoir (6/96)[1]	83*[2]	4
Austin Cary Ponds (8/97)	67*	0
Austin Cary Ponds (5/98)	85*	1

*Differences in sprouting between reduced sediments and distilled water according to a t-test$_{0.05}$

[1]Dates indicate times when studies were initiated

Anoxia has been reported to increase sprouting rates of hydrilla and pondweed tubers (Spencer and Ksander 1997), however, this study suggests that anoxia was a requirement for this sub-population of hydrilla tubers Differences in sprouting requirements may allow a small fraction of tubers to remain quiescent under conditions which generally stimulate a large proportion of the propagules to sprout. For example, drawdowns often stimulate tuber sprouting (or rotting) in excess of 85%, however, explanations for the remaining 15% that remain quiescent have generally focused on the external environment (e g some areas remain wet or reduced, microsite variations etc)

An internal que that prevents tuber sprouting under favorable conditions (i e following a drawdown) may ensure that a small population persists if the sprouted tubers encounter unfavorable conditions and are unable to survive and replenish the propagule bank

Summary and Conclusions

Laboratory studies suggest that removing dioecious hydrilla tubers from the sediment for sprouting evaluations is feasible, however, tubers must be placed in treatment vessels immediately, and the time of year at which *in situ* tubers are collected may dramatically impact the and result. The inhibitory role of CO_2 in these studies was equivocal, and further studies are required to determine if reduced sprouting is due to a physiological or toxic mechanism Hormone studies suggested that effects were similar under both anoxic and aerobic conditions. ABA was a stroog inhibitor of sprouting, and in some cases this effect could be overcome by the addition of GA. Inhibitors of ethylene synthesis and ethylene action only impacted tuber sprouting at the highest concentrations tested Moreover, ethanol did not impact tuber sprouting until concentrations were elevated to a point where the effect was likely narcotic A small sub-population of tubers that are removed from the sediment often display " dormancy", however, this can be overcome by placing these tubers back under anoxic conditions. Techniques to improve research manipulation of the micro-environment of *in situ* tubers is strongly recommended in future studies to evaluate additional information on tuber sprouting.

CHAPTER 6
SUMMARY AND CONCLUSIONS

These studies provided knowledge on the factors that influence population dynamics, sprouting, and quiescence of dioecious hydrilla tubers. Hydrilla is a serious and spreading exotic weed problem in the United States, and the state of Florida is currently spending approximately 10 million dollars annually to control this species. A true maintenance control program has been difficult to attain with hydrilla due to poorly understood factors affecting regrowth following control measures and the high cost of control options. Due to the fact that hydrilla tubers are a primary means of survival and regrowth, a greater understanding of these propagules is key to improved control programs.

Further, much of the research done to date has produced conflicting and variable results which are hard to interpret. Research on hydrilla tubers has been on propagules removed from the anaerobic environment and few if any studies have focused on *in situ* response of tubers following managment.

Vertical distribution of tubers was characterized at sites throughout North Central Florida and subsequent *in situ* sprouting was characterized. Tubers collected from sand dominated sediments had distinct distribution profiles, whereas, tubers collected in soft organic sediments were more randomly distributed. Mesocosm studies designed to determine factors that influence vertical distribution of tubers, suggested that mechanical impedance regulates depth of tuber formation to approximately 20 cm. Nonetheless, no

differences in sprouting potential were noted between tubers located at depths ranging from 3 to 27 cm. Results indicated that there was no environmental gradient that allowed increased tuber sprouting at shallower depths as was hypothesized.

Several researchers had suggested that removal of the vegetative hydrilla canopy from the water surface could stimulate tuber sprouting due to increased sediment temperature, gas exchange, or light penetration. A series of mesocosm studies were conducted to test this hypothesis, as well as others concerning effects of tuber age on sprouting and the duration of a drawdown. Mesocosm studies indicated that canopy removal alone did not stimulate tuber sprouting, whereas, treatments that resulted in death of the root system significantly increased sprouting rates at 8 through 20 weeks after treatment. Further studies demonstrated that increased sprouting rates were also influenced by sediment type and the type of container used. Increased sprouting rates were noted in sandy sediments and in shallow plastic flats. In studies that employed deep PVC cores (tubers and roots were not closely associated), sprouting rates were similar for all treatments. These results suggested that the artificially close association between roots and tubers exists in the shallow plastic flats stimulated tuber sprouting. It is hypothesized that this increased sprouting was caused by changes in the redox potential of the microenvironment of the tuber in the flats. These mesocosm studies also indicated that tuber age had no impact on the likelihood for sprouting following various management techniques. In sand and loam sediments, a drawdown period as short as 24 hours resulted in a significant change in redox potential that was correlated with an increase in tuber sprouting at 60 DAT.

Field studies to determine sprouting and population dynamics of hydrilla tubers showed that sprouting was independent of herbicide or grass carp treatment. Sprouting rates generally remained quite low (<3%), with a peak generally noted between September and November (6-10%) in all ponds. Tuber viability remained greater than 90% through 30 months posttreatment. Vegetative growth in ponds treated with the herbicide fluridone did not readily recover from treatment although sprouting tubers were collected at each sample period. Subsequent analysis of sediment pore water indicated sprouting tubers were exposed to threshold concentrations of fluridone (3 to 6 μg/L) prior to emergence. Sprouted tubers did not establish in these ponds presumably due to the herbicidal effects of fluridone in the pore water. Although tuber numbers increased over time in untreated ponds, values were much lower than would be expected due to the high level of vegetative biomass that was present. Rootcrown density in both mesocosm and pond studies decreases over time due to intraspecific competition. As rootcrowns serve as loci for tuber production, reduced density decreases overall tuber production as well as increases the spatial heterogeneity of tubers. In ponds where hydrilla was allowed to recover in June 1998, new tuber production was prolific by November 1998. The new infestations of hydrilla resulted in high rootcrown density and replenishment of the tuber bank to near pretreatment levels in just 2 to 3 months.

The majority of studies that have evaluated sprouting of hydrilla tubers have relied on removing the tubers from the sediment which has resulted in sprouting rates exceeding 90%. While several of these studies have identified compounds that increase sprouting rates, very few have focused on treatments that inhibit sprouting. The current research suggests that the length of time a tuber is removed from the sediment (>0.5 hr)

increases the likelihood of sprouting. Tubers removed from the sediment and then immediately placed back in sediment, sprouted at rates similar to undisturbed tubers. One exception to this observation was for tubers collected in August. These tubers generally sprouted in relation to disturbance regardless of the length of time they remained removed from the sediment. The high sprouting rates for tubers collected in August suggest that at this time of the year tubers may be predisposed to sprout if properly stimulated. Overall these results suggest that tubers respond rapidly to an external stimulus (i.e. removal from the sediment), and once initiated to sprout this response is generally irreversible. Laboratory studies suggest that abscisic acid (ABA) at 0.05 to 1.0 μM was a strong inhibitor of tuber sprouting under both aerobic and anoxic conditions. In some cases this inhibition could be overcome by addition of GA_3 (15 to 150 μM). As previous research has demonstrated a role for ABA/GA in tuber formation, these studies suggest a similar relationship for tuber sprouting. Inhibitors of ethylene action and synthesis, as well as ethanol (product of anaerobic metabolism) did not impact tuber sprouting at physiological rates.

It is suggested that future research focus on methods to improve measurement of redox conditions in the direct vicinity or microhabitat of the tuber. While the current studies provide extensive evidence to suggest tubers sprout in relation to changes in sediment redox or oxygenation, experimental determination of this phenomenon in systems that remained flooded was elusive due to the microscale (root/tuber association) at which these changes likely occur. To overcome problems associated with blind sampling for *in situ* tubers, experimental systems that allow visualization of the tuber and techniques that allow micromanipulation and analysis of the microenvironment are

suggested In addition, studies to determine changes associated with ABA content of tubers following their removal from the sediment may yield information on the role this hormone plays in *in situ* sprouting.

A better knowledge of tuber population dynamics will be critical to improved maintenance control programs as hydrilla continues to expand and spread throughout the United States Past research on hydrilla tubers has produced conflicting results that are difficult to interpret. For example, tubers removed from *in situ* conditions usually sprout at rates greater than 90 percent, whereas those remaining *in situ* sprout at rates of less than 5% While previous research has suggested that hydrilla is capable of producing greater than 2000 tubers/m^2/year, this study suggests that after 20 years of hydrilla infestation in the field, a rather stable tuber population exists In contrast, due to high rootcrown densities and limited intraspecific competition, new infestations may have potential to rapidly produce large numbers of tubers that further complicate hydrilla management decisions

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BIOGRAPHICAL SKETCH

Michael D Netherland was born on November 16, 1963, in Huntington, Indiana. He grew up in Lawton, Oklahoma, and developed an interest in aquatics at an early age. He graduated from Eisenhower High School in 1982 and received a Bachelor of Science degree in biology from Cameron University, Lawton, Oklahoma, in December 1986

In January 1987, he entered graduate school at Purdue University, West Lafayette, Indiana, and did his graduate work on aquatic invasive plants He received a Master of Science degree in botany in May 1989. Upon graduating, he accepted a job with the U S Army Engineer Waterways Experiment Station in Vicksburg, MS, as a research biologist His work focused on methods to improve herbicide efficacy and reduce use rates

In August 1995, he enrolled at the University of Florida to pursue a doctor of philosophy degree under Dr William T Haller Recently he accepted a position with the SePRO Corporation as Director of Aquatic Research He is a member of The Aquatic Plant Management Society, The Weed Science Society of America, and Sigma Xi

Michael D Netherland is married to the former Miss Marci Love of Lawton, Oklahoma. They have two children, Luke and Sarah.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

William T Haller, Chair
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

George Bowes
Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Donn G Shilling
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Randall Stocker
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

David L. Sutton
Professor of Agronomy

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

May 1999

Dean, College of Agriculture

Dean, Graduate School